The structuring of aquatic macroinvertebrate communities within cave streams

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A thesis

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Dedicated to my parents,

who introduced me to the world and wilderness

Abstract

The unusual environmental conditions within caves provided unique opportunities for developing an understanding of ecosystem processes. However, relatively few studies have been conducted on the ecology of New Zealand cave systems. The primary aim of this research was to investigate changes in aquatic invertebrate communities along a longitudinal gradient from the surface into caves and investigate the fundamental drivers of cave communities. This study was carried out in three streams (two in pasture catchments and one in a forested catchment) flowing into caves in the Waitomo region, North Island, New Zealand. In order to address these aims I carried out a longitudinal survey of 12 sites in each stream, an experimental manipulation of food, and an isotopic study of a single stream. The longitudinal survey of the three cave streams revealed light intensity as well as algal and CPOM biomass all decreased significantly from outside the caves into caves. In contrast, water temperature, dissolved oxygen, stream width, depth, and velocity did not vary significantly with distance into caves. Benthic aquatic macroinvertebrate communities within the caves were a depauperate subset of surface communities, appearing to be structured by gradients in resources and colonisation through drift. However, some invertebrate taxa (primarily predators) were rarely found within caves, further suggesting that resource gradients were structuring cave communities. Surprisingly, the densities of some collector-browsers (primarily mayflies) increased within cave streams relative to surface densities. This may be due to a decrease in competition and predation, flexible feeding strategies, and high drift propensity. However, the benthic densities of most taxa within the caves appeared to be related to drift densities. Although surface forest and pastoral stream communities differed in community composition and density 32 meters within the caves invertebrate community diversity and density became similar, although specific taxa within communities varied. This convergence was attributed to similar environmental gradients within the caves. The resource addition experiment (adding leaf packs) indicated that cave streams were resource limited; the addition of leaves produced communities of similar richness and density across the environmental gradient. The isotopic survey suggested cave stream invertebrate communities were reliant upon similar basal resources to surface streams. However, within the cave epilithon appeared to be increasingly important while filamentous algae were absent. Cave

aquatic invertebrates were also found to support terrestrial predators (spiders, harvestmen, and glow-worms), presumably increasing the abundance and diversity of terrestrial cave communities. In conclusion, aquatic cave communities were reliant upon surface derived resources and consequently strongly linked to surface land-use and managerial practices.

Contents

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Secondly, this research would not have been possible without the permission and goodwill of the land owners. On this note I would like to thank the Murchie, Geange, and Osbourne families who put up with someone with a weird assortment of gear walking randomly around their properties looking for holes in which to disappear. A special thanks to the Osbourne's who turned the bulls (who would chase me daily, scattering temperature loggers, invertebrate samples, lights, and the accursed tanglefoot across the landscape) into hamburgers.

Early one morning in my second year of university Dad woke me up to say there was a scientist going into the cave at work and I should tag along. In a car park standing in a wet wetsuit I met Mike Scarsbrook from NIWA and mumbled that I wanted to do a MSc in a few years time. We went back to my place for a cup of tea and a chat, consequently found the milk was off and there was no tea because Mum was in Australia, and decided to go caving

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Entrance to Murchies cave

Chapter 1

General introduction: the structuring of aquatic macroinvertebrate communities within cave streams

Introduction

The fundamental difference between cave and surface ecosystems is the presence of light (Barr 1967, Azad and Borchardt 1969, Death 1989, Simon and Benfield 2001, Simon et al. 2003, Wood et al. 2008). Light drives autochthonous food-webs through photosynthetic fixation of carbon (Azad and Borchardt 1969), accounting for the majority of aquatic primary production on earth. The exceptions being a few chemotrophic systems recorded in deep-sea vents and caves (Sarbu et al. 1996). Therefore, surface stream communities abundant in light should differ markedly from cave stream communities in their sources of energy (Barr 1967, Howarth 1983, Culver et al. 1995, Gunn et al. 2000, Simon and Benfield 2001, Simon et al. 2003, Moseley 2007, Tobler 2008, Wood et al. 2008, Roldán and Hernández-Mariné 2009). Cave communities are primarily dependent upon surface derived allochthonous and detrital resources, received via water and air flow, animals, and root exudates (Polis et al. 1997). Reliance upon surface originating detritus intimately links cave streams and their associated invertebrate communities to surface streams (Death 1988, 1989, Simon et al. 2003, Wood et al. 2008). The aim of my study was to quantify changes in aquatic invertebrate communities that occur as surface streams flow into caves and investigate the fundamental drivers of cave ecosystems. In this chapter I will briefly discuss the properties of caves, then highlight aspects surface stream ecological theory which might be relevant to caves, and finish with some cave specific examples.

Caves formation and structure

Caves are commonly found in karst, more commonly known as limestone or marble, regions which constitute about 25 percent of the world's land mass (LaMoreaux et al. 1997). For a cave to develop in karst Le Grande (1973) notes that five conditions must occur; 1) the

presence of soluble rocks, 2) the presence of carbonic acid, 3) ample precipitation, 4) openings in the rocks, and 5) a favourable topographical and geological structure.

Karst terrains are composed of limestone (calcium carbonate), or its metamorphosed form marble, which is readily dissolved by weak carbonic acids formed in rain and soils (Le Grand 1973, Williams 1992). Carbon dioxide laden water sinks through surface cracks dissolving and eroding as it percolates to the water table - leading to a paucity of surface streams (Le Grand 1973, Williams 1992). Thus, surface cracks become enlarged and a complex system of vertical and horizontal cracks forms. Larger cracks capture a greater portion of the sinking water and are enlarged even further until the water table drops, or the source of the water is diverted and a dry passage is created (Howarth 1983).

Distinctive features of karst terrains, besides the scarcity of surface streams, include; thin soils, and rugged topography. The high permeability of karst landscapes intricately links surface and cave environments (Howarth 1983, Williams 1992, Bolstad and Swank 1997, Gunn et al. 2000). Linkages occur through multiple fissures, allowing surface sediments and nutrients to seep into caves, especially during precipitation events (Howarth 1983, Bolstad and Swank 1997, Gunn et al. 2000). Thus, surface land-use is liable to significantly influence cave communities (Gunn et al. 2000, Wood et al. 2002).

Subterranean environments present a number of unique conditions for organisms. They are characterised by a labyrinth of dark passages, relatively constant temperatures (near the average annual surface temperature), a moist substrate, a water saturated atmosphere, and decreasing contact with the surface environment (Howarth 1983). Typically the number and density of species diminishes rapidly just inside of the cave entrance, as many species are intolerant of the cave conditions (Death 1988, 1989). A thorough review of basic cave structure and physical and environmental conditions is provided by Howarth (1983), and for Karst landscapes within New Zealand Williams (1992).

General stream ecological theory

Cave communities offer an opportunity for investigating community dynamics (e.g. specific stress gradients), this is because of their relatively depauperate communities, well defined boundaries, and relatively constant environmental conditions (Gibert and Deharveng 2002).

Stress gradients

Resource gradients are one of the primary environmental factors which alter community composition and functioning (Peckarsky 1983). In the context of this study the lack of light driven food resources is the most likely potential stressor moving the cave system towards the harsh end of a harsh-benign environmental gradient (Figure 1.1). Under this model, harsh environments, such as caves, are increasingly structured by abiotic factors. For example, light gradients, while more benign surface systems are more likely to be structured by biotic factors, such as competition and predation.

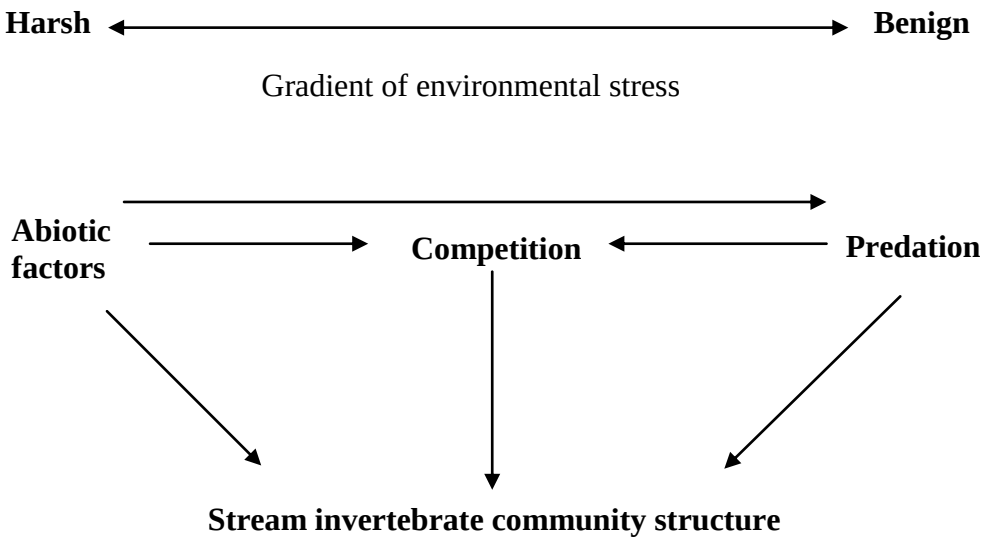

Figure 1.1. Conceptual model of the regulation of stream community structure along an environmental gradient, from Peckarsky (1983).

Effects of flow

Lotic ecosystems are fundamentally regulated by flow levels, velocities, and direction, i.e. downstream communities are linked to upstream processes (Allan and Castillo 2007). Hart and Finelli (1999) remarked that flow effected stream biota in so many different and powerful ways that it should be viewed as the primary environmental factor within lotic ecosystems. Flow influences, but is not limited to, disturbance, the creation of habitats, dispersal (passive or active), predator-prey interactions, competition (inter and intra specific), and resource acquisition (Figure 1.2) (Hart and Finelli 1999).

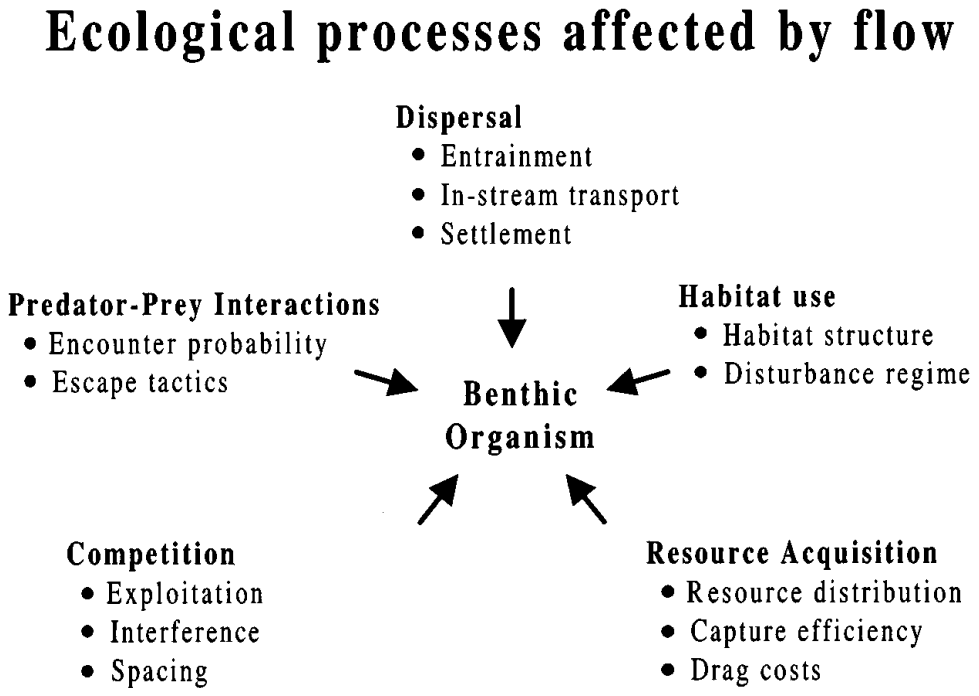

Figure 1.2. Ways by which flow can affect benthic organisms. Key components of each ecological process can be modified by flow, thereby affecting the performance, distribution, and abundance of organisms. Potential interactions among pathway are not shown, from Hart and Finelli (1999)

Dispersal in the drift

The direction of flow also strongly influences invertebrate communities through drift. Drift, occurs when an organism is carried by water currents, and may be passive or active (McLay 1970, Kohler 1985, Death 1989, Hart and Finelli 1999, McIntosh 2000, McIntosh et al. 2002, Duan et al. 2008). Most animals that occur in the benthic fauna are also found in the drift, in relatively proportional abundances. Variations in the proportion arise because of differences in a taxa's behaviour or structure (McLay, 1970), resulting in species assemblages with differing susceptibilities to current (Holomuzki and Biggs 2000). For example, Ephemeroptera are recognised as active drifters (Kohler 1985), while other orders, such as Mollusca, are rarely found in the drift. Holomuzki and Biggs (2000) found that the common New Zealand snail *Potamopyrgus* moved deeper into the substratum to avoid high flows, and dislodgement of individuals did not occur until the substratum was moved. Their results suggested that active drifters such as *Deleatidium* used drift to persist in flood prone streams, whereas behavioural modifications played a key role in snail and caddisfly survival, i.e. a reliance upon cases (Holomuzki and Biggs 2000).

Source sink dynamics

Variations in habitat suitability creates patchy distributions of populations within, or between, ecosystems (Turner 1989), leading to the formation of metapopulations (Pulliam 1988, Leibold et al. 2004, Holyoak et al. 2005). Source-sink dynamics occur between metapopulations when surplus individuals from highly productive source habitats move to less productive sink habitats. In source-sink conditions within-habitat reproduction fails to keep pace with within-habitat mortality, so migrants from source populations prevent sink populations from extirpation (Pulliam 1988, Pulliam and Danielson 1991). Source-sink dynamics can be driven by differences in food and/or habitat availability (Foppen et al. 2000), as well as predation levels (Woodford and McIntosh 2010). Source-sink relationships seem likely between surface and cave environments as caves are likely to have low internal recruitment.

Land use change

Caves are intimately connected to surface systems. Therefore, changes in land-use and management practices within surface catchments can result in significant changes to cave ecosystems (Watson et al. 1997, Poulson and Lavoie 2000, Hancock et al. 2005, Wood et al. 2008). Alterations to catchments include quarrying, agriculture, waste disposal, and groundwater abstraction (Gunn et al. 2000), and have all been shown to effect surface stream health, through impacts on physio-chemical and biotic conditions within aquatic ecosystems (Harding et al. 1999, Young and Huryn 1999, Quinn and Stroud 2002, Collier and Quinn 2003, Death et al. 2003, Larned et al. 2004, Townsend et al. 2008, Wilcock et al. 2009).

Forest versus pasture

One of the commonest land-use changes has occurred in many New Zealand river catchments, the shift from forest cover to open grasslands (Quinn 2000, Ewers et al. 2006). In this thesis I have focussed on comparing cave streams within both forested and agriculture land-uses. Forested streams are typically characterised by heavy shading, and allochthonous inputs, whereas pastoral streams are typically unshaded, highly autotrophic systems (Hicks 1997, Quinn and Cooper 1997, Quinn 2000, Simon et al. 2003). These contrasting systems are based upon different food sources; forested streams rely primarily on detritus from the riparian zone, such as coarse particulate organic matter (CPOM), and minimal autochthonous production. Whereas pastoral streams rely primarily upon autocthonous production (e.g. algal), because of the high availability of light and nutrients and often poor levels of riparian

vegetation (Quinn 2000, Simon et al. 2003). Autochthonous energy is energy produced within streams, e.g. photosynthesis in algae, macrophytes, and biofilms. Allochthonous energy is energy produced outside of streams, e.g. coarse particulate organic matter (CPOM) and fine particulate organic matter (FPOM), and subsequently imported to the stream environment. CPOM is an important direct food source and habitat (Winterbourn 2000), as well as an important indirect food resource for invertebrates, acting as substrate for biofilms (Simon and Benfield 2001, Simon et al. 2003). FPOM is also an important food source for stream invertebrates (Winterbourn 2000), and can be transported long distances, as seston, within the water column (Ock 2010). These sources of energy are vital to stream ecosystems and play a significant role in the structuring of invertebrate communities (Townsend et al. 2004).

In summation, an organism's occurrence in any habitat depends on its ability to disperse to that location and, upon arrival, tolerate local environmental conditions, use resources, and interact competitively with its neighbours. Taxa may persist in a sub-optimal habitat despite extinctions through dispersal from source populations (Pulliam 1988, Leibold et al. 2004, Holyoak et al. 2005). Within streams invertebrate dispersal is generally through drift (McLay 1970, Death 1988, 1989, McIntosh 2000, McIntosh et al. 2002), although it can be facilitated by adult dispersal (Bunn and Hughes 1997, Winterbourn and Crowe 2001).

Cave stream community regulation

When streams enter caves their character and community are the product of their catchments; reflecting the disturbance, resource supply, and competitive interactions dictated by upstream conditions. However, upon entering caves some of these conditions change fundamentally and rapidly, although the degree of change will depend upon their associated upstream characteristics. The invertebrate communities of each cave will reflect the upstream conditions of that stream, changing environmental gradients within the cave, and the supply of colonists (Death 1989).

Cave environment

The cave environment is primarily characterised by a lack of light and increasing environmental stability. There is a constant lack of riparian inputs, stable temperatures, high humidity, and darkness; however flooding is still a common disturbance within caves. At the location of the cave and surface ecotone edge effects may occur (Hansen et al. 1988, Nakano

and Murakami 2001, Simon and Benfield 2001, Hancock 2002, Prous et al. 2004, Hancock et al. 2005). The cave ecotone can be classified into three distinct zones (Figure 1.3). The surface-cave, or para-hypogean, located immediately above the cave entrance, and greatly affected by the surface system. The cave-surface edge, twilight zone or para-epigean, which extends from the entrance until light is lost. Finally, the dark, hypogean zone, beyond the penetration of light, which is increasingly isolated from the surface and increasingly stable (Aley and Aley. 1997, Prous et al. 2004). Ecotones often have different communities and higher diversity to their adjacent ecosystems, because organisms from both environments may inhabit or forage in them (Hansen et al. 1988, Andren 1992, Nakano and Murakami 2001, Prous et al. 2004).

Resource limitation in caves

Previous studies on invertebrates communities in caves have found them to be resource limited with strong links to surface environments (Le Grand 1973, Death 1988, 1989, Gunn et al. 2000, Simon and Benfield 2001, Simon et al. 2003, Wood et al. 2008). Death (1989) found benthic invertebrate densities much lower within a South Island alpine cave, and attributed this to the lack of food, although relative abundances were similar. Death (1989) also noted that there was potential for sustainable benthic invertebrate populations to exist within cave systems, but it would seem more likely that populations of the majority of taxa were strongly influenced by upstream flow and drift. However, the net spinning caddis fly *Aoteasphysche* was present in higher numbers than predicted, which was attributed to their ability to feed upon FPOM in the water column (Death 1989).

Simon et al. (2003) used stable isotope analysis and tracer additions to confirm cave stream reliance upon imported surface material, and noted that caves may represent the heterotrophic extreme in the continuum of stream types. However, they were unable to discern the relative importance of the different fractions of allochthonous material; dissolved organic matter, particulate organic matter, and associated microbes (Simon et al. 2003).

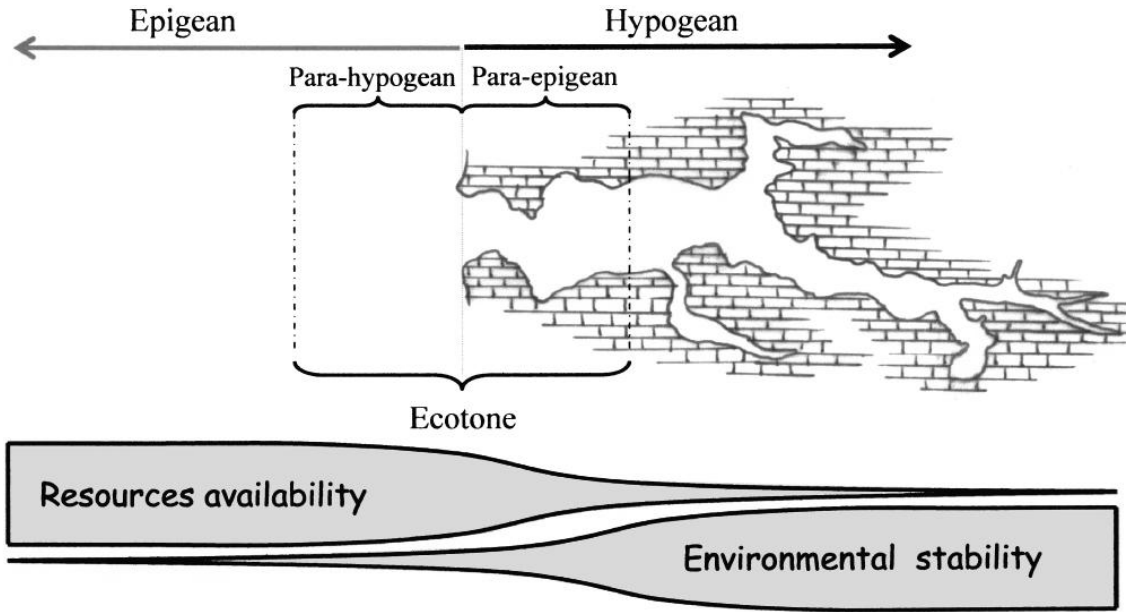

Figure 1.3. The epigean–ecotone–hypogean system. The ecotone is located in a zone characterised by gradients in environmental stability and resource availability. Hypogean and epigean communities are located in opposite extremes – the former in a stable environment with low resource availability and the latter in a zone of low environmental stability with high resource availability. From Prous et al. (2004).

A study by Vinogradova et al. (1998) assessed how the loss of light affected epilithic cyanobacteria diversity, in the dry, 90 m deep, el-Wad cave, in Mount Carmel, Israel. Altogether 42 species from 20 genera of blue-green algae were found. Between the entrance and the end of the cave the number of coccoid forms decreased and filamentous (especially oscillatorial) taxa increased. Vinogradova et al. (1998) concluded that the diversity of species and community composition of cyanophytes was reduced from the entrance to the end of the cave concomitantly with darkness stress.

Cave fauna

Invertebrates show variable affinities to cave environments, which has led to a classification system to describe cave invertebrates. Cave aquatic organisms have been classified into four categories (Gibert et al. 1994).

1) *Stygobites*, cave obligate species.

2) *Stygophiles*, facultative species that live and reproduce not only in caves but also in cool, dark, moist micro-habitats outside of caves.
3) *Stygoxenes*, species that utilise caves, but cannot complete their life cycle entirely in caves.
4) Cave accidentals.

The prefix *stygo* is used in reference to aquatic cave organisms, as opposed to *troglo* which is used to describe terrestrial cave organisms.

In Peak and Speedwell Caverns, England, Gunn et al. (2000) found relatively few stygobiotic taxa, and the majority of taxa were described as stygophiles or stygoxenes. Invertebrate communities in allogenic cave streams, (surface streams entering caves), had a higher diversity than autogenic streams, (streams beginning underground). The majority of taxa within caves were also found in surface streams, although dispersal mechanisms were not always obvious. The shining pea clam *Pisidium nitidum*, was found to inhabit a stream derived from autogenic percolation and the direction of flow was towards the cave entrance, and its mode of colonisation was uncertain (Gunn et al. 2000). Gunn et al. (2000) also noted that epigean populations within the cave were often impoverished, compared to surface communities, suggesting that invertebrates had difficulty in travelling through and/or surviving in karst ecosystems.

Considerable literature exits on obligate cave dwelling species within deep caves. The species that have received the most attention are iconic or cash-species such as the Ozark cave fish, *Amblyopsis rosae*, (Stewart 1984, Aley and Aley. 1997, Graening and Brown 2003, Graening 2005), and glow-worms, *Arachnocampa* spp., within Australia and New Zealand (Pugsley 1984, Broadley and Stringer 2001, Baker et al. 2008, Driessen 2010). *Arachnocampa luminosa* (New Zealand's only currently identified species) are troglophiles, as they also inhabit damp epigean environments. *A. luminosa* live in mucus hammocks suspended from the roof and use bioluminescence to attract prey which they ensnare on sticky mucus threads (Pugsley 1984). *A. luminosa* are known predators of adult stream invertebrates, e.g. mayflies and chironomids, and therefore the continued persistence of these organisms within any cave is partially dependent on the supply of invertebrate prey (Pugsley 1984). *A. luminosa* are generally found in colonies, as their combined light attracts more prey, however territorial competition occurs, and dominant organisms throw weaker individuals from the roof, or

consume them (pers. comm. Professor D. Merrit). Opiliones (Harvestmen) also occupy cave roofs, preying on both adult aquatic insects and glow-worms (Broadley and Stringer 2001). Thus, the terrestrial cave community is structured by the supply of prey resources from streams and competitive interactions within and between populations. At the time of this research there have been no published studies on fish within cave environments, in New Zealand.

The primary aim of this research was to assess differences between the structuring of cave and surface stream macroinvertebrate communities and to determine the main drivers in differences between these communities. To address these questions I undertook a survey of abiotic and biotic factors within caves streams, which is discussed in chapter two. This led to an experimental manipulation of resources (algal, habitat, and CPOM) with respect to invertebrate communities, discussed in chapter three. The fourth chapter covers an isotopic survey assessing energy flow within a cave system, and finally findings were summarised and implications for management of cave systems were discussed in chapter five. The thesis has been structured as a series of standalone scientific manuscripts (with references provided at the end of each chapter), as such there is some repetition between chapters.

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Aoteapsyche 700 meters within Murchies cave

Chapter Two

Cave communities across a longitudinal gradient: source-sink populations structured by resource limitations

Abstract

Caves are unique ecosystems with challenging environmental conditions, resulting in relatively depauperate communities. One of the most important approaches to understanding their invertebrate communities is by investigating their structuring forces. To assess the structure of cave communities, across an environmental gradient, I investigated three streams (two in pasture catchments and one in a forested catchment) flowing into caves in the Waitomo region, North Island, New Zealand. In each stream 12 sites were sampled longitudinally: four sites were upstream of the cave entrance and the remaining eight sites were arranged from the entrance to 256 m inside the cave. A range of physical, chemical, and biological parameters were assessed along this environmental gradient. As expected, light intensity and algal production were tightly linked, high at surface sites and exponentially decreasing within the caves. Cave communities had significantly fewer benthic invertebrate taxa (P = 0.046) and lower densities (P = 0.0020) than surface communities. Cave benthic invertebrate communities were a sub-set of surface communities, primarily structured by a decrease in resources, and surface recruitment. Further than 32 m inside the caves similar invertebrate densities were found, suggesting that resources were constant and a continuous supply of invertebrates, via drift, meant resources were exploited. The propensity of invertebrates to drift increased within the caves, presumably because caves were resource limited, while drift density remained constant. Several taxa were found at densities varying from those expected, suggesting that the physical and behavioural adaptations of taxa play a role in survival. Finally, caves constituted a sink, arising from the surface stream invertebrate source populations, further confirming the importance of upstream surface land-use and stream health.

Introduction

Globally, 97% of all unfrozen freshwater is subterranean, surface lakes and rivers encompass less than two percent (Gibert et al. 1994). Despite the importance and volume of subterranean waters, surprisingly little ecological research has been conducted on these systems, and even fewer studies have intensively investigated aquatic communities and food-webs within caves (Simon et al. 2003). Cave streams experience a different set of environmental conditions, relative to those occurring on the surface. They include an absence of light, limited temperature variation, and scarcity of food, resulting in caves providing extreme, harsh, habitats for aquatic life (Ginet and Decou 1977). Abiotic and biotic gradients drive changes in resource availability and quality (Howarth 1983, Culver and Sket 2000, Gunn et al. 2000, Simon et al. 2003, Tobler 2008), as well as competitive interactions within and between species (Tobler 2008). As a result, caves tend to sustain depauperate, weakly productive communities (McMullin et al. 2000, Tsurumi 2003, Tobler 2008).

The unusual environmental conditions within caves provide unique opportunities for developing an understanding of ecosystem processes, and functioning (Gibert and Deharveng 2002). Specifically, caves provide excellent models of how/if changes in biodiversity and resources, such as energy and habitat, affect invertebrate communities (Ginet and Decou 1977, Death 1988, 1989, Sarbu et al. 1996, Looser et al. 1999, Gunn et al. 2000, Simon et al. 2003, Graening 2005, Herrando-Perez et al. 2008)

The most obvious difference between surface (epigean) and cave habitats (hypogean) is the lack of solar energy in caves. In surface environments this elemental building block of life fuels the evolution of complex diverse communities (Azad and Borchardt 1969). However, because cave communities are reliant upon limited amounts of detrital energy they tend to have relatively simple, depauperate communities (Gunn et al. 2000). Thus, hypogean and epigean communities can be viewed as representing opposite extremes of a continuum, with epigean communities being structured by high resource availability, and hypogean communities being structured by low resource availability (Death 1989, Simon et al. 2003, Prous et al. 2004).

A major factor limiting aquatic communities in caves is therefore the supply of carbon, with the majority of cave systems entirely dependent on allochthonous, detrital resources (Barr 1967, Simon and Buikema 1997, Looser et al. 1999, Gunn et al. 2000, Wood et al. 2002,

Simon et al. 2003, Wood et al. 2008). There are few examples of autochthonous energy sources in caves, such as those provided by chemotrophs (Sarbu et al. 1996, Engel 2007, Porter et al. 2009). Sarbu et al. (1996) studied a cave system in Romania that had a water temperature of 21^{o}C and was rich in hydrogen sulphide. Chemoautotrophic energy provided the trophic basis for the community, which had many similarities to deep-sea volcanic communities (Sarbu et al. 1996). However, apart from rare examples such as this, caves are thought to be primarily dependent upon surface-derived materials.

The point at which ecosystems or habitats meet is known as an ecotone (Prous et al. 2004) (Figure 1.3). This transition zone between adjacent ecological systems has a set of unique features defined by spatial scale, and the strength of the interactions between the systems (Hansen et al. 1988). Within an ecotone diversity can be higher, similar, or lower than in adjacent ecosystems (Prous et al. 2004). Cave ecotones can be divided into distinct zones. First, the entrance, or para-hypogean zone is located immediately outside the cave mouth, and is greatly affected by the surface system. The twilight, or para-epigean, zone extends from the entrance to a point where light is lost. Finally, the dark, or deep cave zone is almost entirely isolated from the surface (Aley and Aley. 1997). The response of stream communities to these transition zones has not been documented in New Zealand.

Terrestrial land-use activities such as agriculture, quarrying, and waste disposal have significant impacts on physico-chemical and biotic interactions within surface aquatic ecosystems (Quinn and Cooper 1997, Harding et al. 1999, Quinn 2000, Quinn and Stroud 2002, Collier and Quinn 2003, Townsend et al. 2004, Townsend et al. 2008), and they in turn influence the aquatic systems of caves (Gunn et al. 2000, Mazina et al. 2009, Shi et al. 2009). Hence, changes in land-use and management practices in epigean catchments can result in significant changes to hypogean ecosystems (Watson et al. 1997, Poulson and Lavoie 2000, Hancock et al. 2005).

Forested streams are typically characterised by heavy shading and heterotrophic inputs, whereas pastoral streams are typically unshaded, highly autotrophic systems (Figure 2.1) (Hicks 1997, Quinn and Cooper 1997, Simon et al. 2003). These contrasting systems are based upon different food (carbon) resources, with forested streams relying mostly on detritus, such as coarse particulate organic matter (CPOM), because of minimal autochthonous production. In contrast, pastoral streams rely upon allochthonous production

(e.g. algal) because of a lack of riparian vegetation and increased nutrient and light levels (Scarsbrook and Townsend 1993, Quinn 2000, Scarsbrook et al. 2001, Niyogi et al. 2007a). Simon and Benfield (2001) noted that nutrient limitation was unlikely to occur within cave streams because nutrient concentrations were typically high in karst groundwater, a result of agricultural inputs. Additionally, cave systems are vulnerable to land-use change as sediments and nutrients readily percolate through fissures in overlying bedrock, particularly during precipitation events (Bolstad and Swank 1997).

Figure 2.1. A typical surface stream within agricultural land, largely unshaded with direct nutrient inputs. Downstream, the stream enters a cave, riparian vegetation is still present, but the stream now flows along a bedrock channel. Within the cave the stream is highly channelised and dominated by bedrock. The dark substance on the bedrock is particulate organic matter. Photos are +128 m, 0 m, and -16 m, from Murchies cave entrance.

Benthic invertebrate community structure within caves can be altered significantly by organic pollution (Wood et al. 2008). In particular, obligate cave dwelling organisms (stygobites) are frequently reduced in number or absent, partially because of the addition of surface nutrients that enable surface stream communities to persist (Culver and Holsinger 1992, Simon and

Buikema 1997, Graening and Brown 2003). Epigean invertebrates are generally not adapted to survive and reproduce within cave environments so the occurrence of epigean animals within cave environments may be indicative of source-sink dynamics. A source-sink interaction occurs when individuals from a highly productive source habitat move into less productive sink habitats where mortality is greater than reproduction (Pulliam 1988, Pulliam and Danielson 1991, Leibold et al. 2004, Holyoak et al. 2005, Woodford and McIntosh 2010).

Benthic cave invertebrate communities may initially be structured by drift from up-stream (Death 1989), but may also respond independently to resource gradients within caves (Simon and Buikema 1997). Drift is a low-energy means of migration, which enables stream invertebrates to leave risky habitats or escape predation (McLay 1970, McIntosh 2000, Winkelmann et al. 2008). The proportional occurrence of species drifting is expected to be more or less similar to that on the benthos, deviations being a consequence of behavioural or structural attributes of the species (McLay 1970). Drift can be passive or active, with active drifters such as mayflies, drifting further than passive drifters, such as cased caddisflies (McLay 1970, Duan et al. 2008, Winkelmann et al. 2008).

In this chapter I report the results of an investigation into longitudinal gradients in invertebrate communities and abiotic variables across cave ecotones. I proposed that surface streams entering caves would undergo marked changes in physical structure and water quality. Firstly, light levels would be reduced at cave entrances, and at some distance into a cave light would no longer be present, resulting in marked changes to primary production (e.g. algal and macrophyte growth). It is also logical to expect that diel variations in light, and therefore water temperature, would affect water temperatures inside the caves. Therefore, I predicted that temperature variations would be greater outside the cave (Figure 2.2a). I predicted that algae would be the main food source for benthic invertebrates outside caves, while inside caves, imported carbon in the form of FPOM (fine particulate organic matter) and CPOM, should become increasingly important. Finally, I predicted that changes in food sources would lead to major changes in benthic communities, particularly taxonomic richness, density, and feeding strategies (Figure 2.2b). However, these changes may not be linear as ecotone effects would be expected to occur around cave entrances and deeper transition zones.

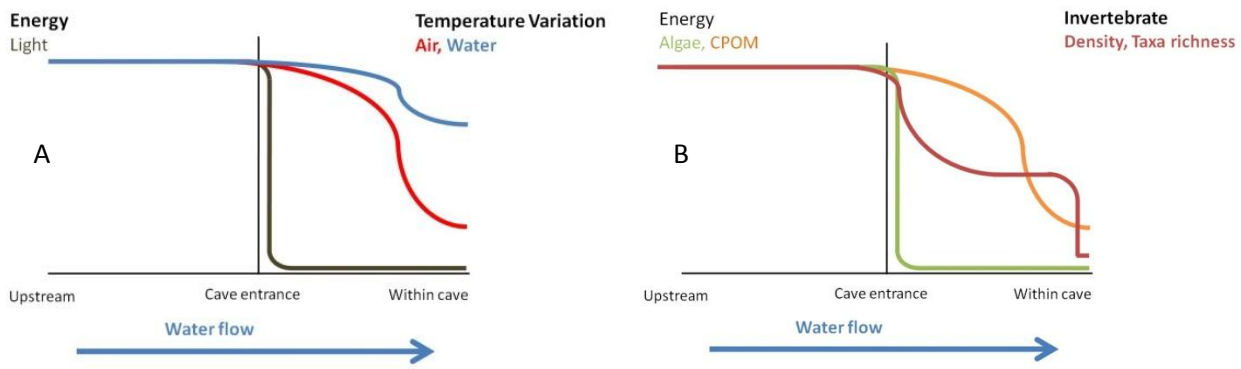

Figure 2.2. A) Predicted variance in light and temperature levels inside and outside a cave. The y-axis shows magnitude of variation, with higher variation occurring at the top. B) Predicted energy source availability leading to a change in benthic invertebrate community. The y-axis represents density, with higher densities occurring at the top.

Materials and methods

Study streams

The study was conducted in three streams (Murchies, Hollow Hill, and Whites) located in the Waitomo region, central North Island, New Zealand. Waitomo is predominantly hilly, with sheep and beef farming common and with fragments of native forest, comprising podocarps, ferns, and hardwood. Geologically the area is dominated by karst (Williams 1992).

Murchies Stream is located 7.8 km from the town of Waitomo and flows into a limestone cave approximately 1 km long (1785080 E, 5767710 N). The catchment is approximately 1.5 km^2 and predominantly covered in native podocarp forest. The stream is second order and flows through approximately 0.95 km of sheep and beef farmland before entering the cave. Murchies cave is characterised by a series of riffles, runs, and pools overlying limestone bedrock substrate.

Hollow Hill Stream is 15 km from the town of Waitomo and is approximately 0.9 km long (1776910 E, 5765940 N). The catchment is approximately 5 km^2, and although the headwaters are dominated by native forest the lower part of the stream flows through approximately 3.3 km of sheep and beef farmland before entering the cave. The stream is second order with riffles, runs, and pools and cobble and bedrock substrate.

Whites Stream is 17.3 km from the town of Waitomo and has a limestone cave approximately 1.1 km long (1.773340 E, 5762940 N). The catchment area is approximately

1.5 km^2, predominantly covered by native podocarps. The stream has two tributaries, one of which is sourced from a farm pond and wetland and one, the larger, from native forest. The stream is composed of riffles, runs, and pools with a limestone bed and silt filled pools.

Study reaches

On each stream 12 sites distributed on a log_2 scale were sampled. Of these, four sites (+128m, +32m, +8m, +4m) were upstream of the cave entrance, one was located at the cave entrance (0 m), and a further seven were located inside the cave (-2 m, -4 m, -8 m, -16 m, -32 m, -128 m, -256 m). At Hollow Hill a rock fall occurred at -16 m so a site was established at -64 m.

A range of physical, chemical, and biological parameters were measured at each site. Air and water temperature loggers (DS1921 iButton Temperature Dataloggers, Maxim Innovation, or hobo data loggers, Onset computer corporation, H08) were placed at seven sites (+128 m, +8 m, 0 m, -8 m, -32 m, -128 m, and -256 m) within each cave. Air and water temperature were measured every 15 minutes from 1 March to 18 March 2009. At each site wetted width was measured and water velocity was calculated at five evenly spaced distances across the stream, using a pygmy current meter. Stream depth measurements were taken at each water velocity location. Spot measures were taken of conductivity, temperature, and pH (Elemetron CX-401- waterproof).

Chlorophyll-*a* biomass was estimated from ceramic tiles (~24.95 cm^2) which were allowed to colonise for an average of four weeks. Six tiles were placed at each of nine sites in each stream (+32 m, +8 m, +4 m, 0 m, -2 m, -4 m, -8 m, -16 m, and -32 m). After four weeks, each tile was removed, scrubbed with a wire brush in water, and the slurry filtered through Whatman glass microfiber filters (diameter 47 mm, nominal pore size 1 µm), wrapped in aluminium foil and frozen. Chlorophyll-*a* was measured using a flurometer (Trilogy 7200-00, Turner designs, Sunnyvale, CA). To extract chlorophyll-*a*, 50 ml pottles (covered with black duct tape, to reduce light) were used. Pottles were rinsed twice with 50% ethanol before the addition of algal samples and were left in a darkened room to extract in 20 ml of buffered ethanol for 12 to 16 hours. Buffered ethanol was made using 90% ethanol and 10% supersaturated magnesium carbonate ($MgCO_3$) solution, which was made by adding 10 g of $MgCO_3$ to 1000 ml of nanopure water, and letting it settle for 24 hours. Three ml of extracted sample was used to rinse test tubes (12 mm by 70 mm glass test tubes), before 3 ml of sample was added to each tube with a micro-pipette. Samples were then measured flurometrically, in

relative fluorescence units (RFU). Immediately after this 30 µL of 0.1 N HCl was added to each test tube and the sample was left to degrade for 2-5 minutes. They were then remeasured to enable chlorophyll-*a* and pheophytin-*a* to be separated. If an RFU value > 1 million was obtained samples were diluted. For diluted samples 0.3 ml of extracted sample was added to the test tube, followed by 2.7 ml of buffered ethanol. The samples were then processed as above. To prepare 0.1 N HCl acid 8.5 ml of concentrated hydrochloric acid was added to 800 ml of deionized water in a one litre volumetric flask, and then topped up with deionized water.

The amount of chlorophyll-*a* in samples was calculated using the following equations:

(a) Chlorophyll *a* per unit area:

Chl *a* (mg / units of area, e.g., m^2) = R * (fluoresence before - fluoresence after) * (extract volume of the sample / area of sample m^2) * (total volume / subsample volume)*(1 / dilution factor)

(b) Chlorophyll *a* per unit volume:

Chl *a* (mg / L) = R * (fluoresence before - fluoresence after) * (extract volume of the sample / total volume) * (total volume / subsample volume) * (1 / dilution factor)

The dilution factor was determined as: dilution factor = volume of sample added to cuvette / (volume of sample added + volume of buffered ethanol added), i.e., the total volume is in the denominator, or the part to the whole). All volumes are in litres. References for chlorophyll *a* extractions methods and equations include Nusch (1980) and Sartory and Grobbelaar (1984).

Samples of fine particulate organic matter (FPOM) in suspension were obtained from each site by taking a 4.5 L water sample, which was filtered through a Whatman glass microfiber filter (diameter 47 mm, nominal pore size 1 µm), wrapped in aluminium foil and frozen. In the laboratory, samples were dried at 45ºC for at least 56 hours. Samples were weighed and ashed at 550ºC for 3 hours. Ashed samples were re-weighed and the ash-free dry weight (AFDW) of FPOM calculated. Coarse particulate organic matter (CPOM) was collected from benthic invertebrate Surber samples, which were kept in 90% ethanol prior to processing. In

the laboratory, organic matter was removed and dried at 45ºC for at least 101 hours. The samples were weighed, ashed at 550ºC for 3 h, re-weighed and the AFDW of CPOM calculated.

Benthic invertebrates were collected from each site in March 2009. Three Surber samples (0.0625 m^2, mesh 500 µm) and one composite kick-net (mesh 500 µm) were taken from each site. All samples were preserved in 90% ethanol in the field, and sorted in the laboratory under a Wild M3 dissecting microscope, at 6.4 to 40x magnification. Identifications were made using the keys and guides of Winterbourn (1973), Chapman and Lewis (1976), and Winterbourn et al. (2000). Identifications were carried out to the lowest taxonomic level possible, except for Chironomidae, which were not separated below tribe.

Benthic invertebrate drift was measured once over 24 h using three drift nets (net opening 105 x 55 mm, mesh 500 µm). Drift was measured at all 12 sites, but because upstream nets might disrupt catches at downstream sites only one site per stream was sampled over any 24 h period. Nets were placed at randomly selected sites and were moved to other randomly selected sites until all 12 sites had been sampled. Water velocity was measured for 90 seconds at the mouth of each drift net, using a pygmy current meter. Drift sampling was carried out during March 2009 when streams were close to base flow, and before benthic sampling began. Samples were transferred to pottles in the field and preserved in 90% ethanol. Drift propensity was calculated by dividing invertebrate drift density by invertebrate benthic density (McIntosh et al. 2002).

Adult flying insects were sampled using six sticky traps placed above the drift nets at each site. Sticky traps were positioned as close to the stream surface as possible, and were constructed from A4 sized sheets (623.7 cm^2) of clear plastic, and coated with Tanglefoot (Tanglefoot CO. USA). Sheets were placed back to back so that three sheets faced upstream and three faced downstream, and left for approximately 24 h, removed, wrapped in "Gladwrap", and frozen.

Statistical methods

Data were transformed when necessary, either by a ln, log, or ln (x+1), to correct for normality (Quinn and Keough 2002). Non-metric Multi-Dimensional Scaling (NMDS) models were run in Primer 6.1.12 (Clarke and Warwick 2001) as were associated analyses of similarity (ANOSIMS). NMDS and ANOSIM were used to compare community composition of the streams. Data from the three caves were analysed separately because their separation in ordination space, suggested different processes and communities occurred in them. Had the combined data set been analysed the results would have described differences between caves rather than patterns common to all caves.

Detrended Correspondence Analysis (DCA) models were run in CANOCO 4.5 (ter Braak and Šmilauer 2002) and de-trended by segments. Data were then log(x +1) transformed and rare species were down weighted. Canoniacl Correspondence Analysis (CCA) models were run in CANOCO 4.5. Data were then log(x +1) transformed and rare species were down weighted. Significant variables were selected manually using 999 Monte Carlo permutations. The significance level was set at $P < 0.10$ to reduce the likelihood of rejecting biologically relevant variables (Legendre and Legendre 1998). An RDA was run for Whites cave, rather than a CCA, because of its low gradient score. RDA models focus on inter-species correlations with species scores divided by the standard deviation. Data were then log(x +1) transformed and rare species were down weighted. Data was centred by species. Significant drivers were selected manually using 999 Monte Carlo permutations. The significance level was set at $P < 0.10$ to reduce the likelihood of rejecting biologically relevant variables (Legendre and Legendre 1998). Permutations were set to unlimited.

Standard errors of the mean were calculated by dividing standard deviations by the number of samples. Regressions were linear and calculated in Sigma plot (2001, Systat Software Inc).

Results

Abiotic conditions

Light intensity, chlorophyll-*a* concentration, and CPOM biomass were significantly correlated with distance from cave entrances ($R^2 = 0.91$, $F = 99.23$, $df = 11$, $P < 0.001$), ($R^2 = 0.84$, $F = 37.45$, $df = 8$, $P < 0.001$), and ($R^2 = 0.47$, $F = 8.99$, $df = 11$, $P = 0.013$), respectively (Figure 2.3). The large variation in light intensity outside caves was influenced by

differences in riparian vegetation density. The length of the twilight zone (distance light penetrated inside the cave) varied between caves, ranging from 8 m to 32 m. The para-epigean zone (immediately above the cave entrance) was less well defined, but was approximately 0 - 8 m. In contrast to light, water temperature, dissolved oxygen, and stream width, depth, and velocity did not vary significantly ($P > 0.05$) between the outside and inside of caves. Unfortunately, FPOM values for all sites did not differ from blank control filters, suggesting that 4.5 L water samples were not large enough to detect FPOM biomass.

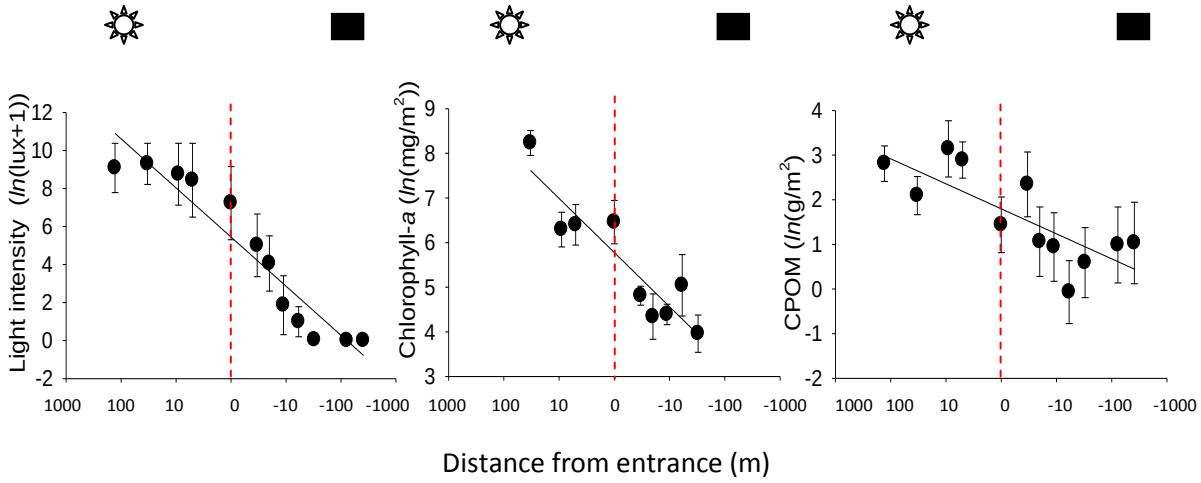

Figure 2.3. Light intensity, chlorophyll-*a*, and CPOM at 12 sites on a longitudinal gradient from upstream to inside caves. Mean ±1standard error (n = 3). In this figure and the following (unless otherwise stated) the dotted line represents the cave entrance, the sun represents outside the cave, and the black square represents inside the cave.

Benthic invertebrate communities

Comparisons of benthic invertebrate taxonomic richness across all three streams showed that richness decreased in caves ($R^2 = 0.34$, $F = 5.19$, $df = 11$, $P = 0.046$) (Figure 2.4). Invertebrate density showed a similar pattern, and was significantly higher outside the caves ($R^2 = 0.63$, $F = 17.08$, $df = 11$, $P = 0.0020$) (Figure 2.4). No aquatic invertebrate species were found to be unique to caves, and as such were a subset of surface communities, upstream of the caves.

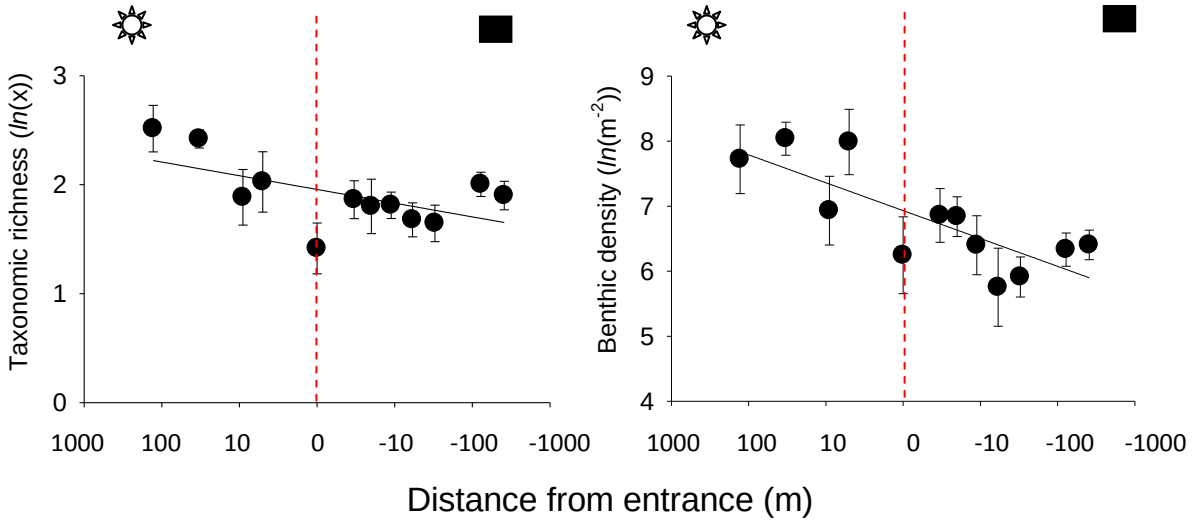

Figure 2.4. Benthic taxonomic richness and density at 12 sites along a longitudinal gradient from 128 m above the cave entrances to 256 m inside the caves. Mean ± standard error (n = 3)

The propensity of invertebrates to drift differed inside and outside the caves (Figure 2.5) and, there was a significant increase in drift propensity (drift density / benthic density, m^3s^{-1}/m^2) with distance into the cave (R^2 = 0.62, F = 16.57, df = 11, *P*= 0.0022). Invertebrate drift density did not differ inside and outside the caves (*P* > 0.05) (Figure 2.5).

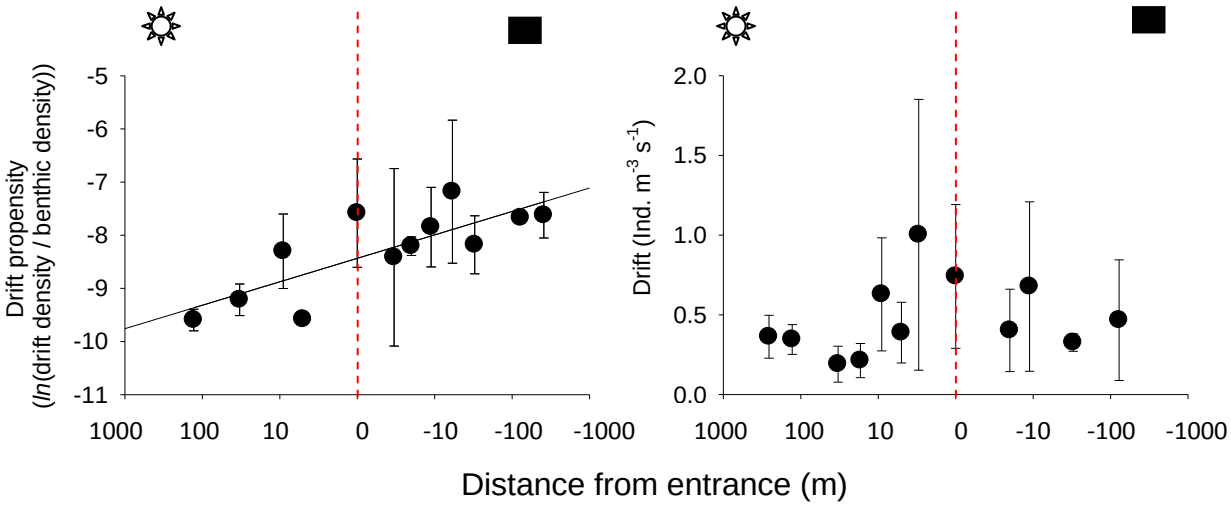

Figure 2.5. Invertebrate drift propensity and density (all species combined) in the three caves. Mean ±1 standard error (n = 3). Where error bars are not shown they were smaller than the symbol.

Individual taxa densities and drift rates varied between surface and cave sites, as well as between caves (Table 2.1 and 2.2.). Mayflies and *Austrosimulium* were more abundant within than outside Murchies and Whites cave, whereas *Aoteapsyche* was more abundant inside Hollow Hill cave than outside it although numbers were small. All other taxa decreased within the three caves, relative to surface sites. *Hudsonema*, *Hydrobiosis* spp., *Oxyethira*, Chironomidae, and Elmidae were absent, or present in very low abundance within the caves, relative to surface sites. Drift densities of mayflies, *Megaleptoperla*, *Hudsonema*, and Elmidae increased within caves, but drift densities of all other taxa decreased within the caves, relative to surface sites (Table 2.2).

Table 2.1. Average densities (m^{-2}) of the most abundant aquatic taxa at surface (sites +128 m and +32 m averaged) and within the three caves (sites -32 m, -128 m, -256 m averaged). Values in brackets are standard errors. Mur = Murchies, Hh = Hollow Hill, Wi = Whites.

	Mur surface	Mur cave	Hh surface	Hh cave	Wi surface	Wi cave
Deleatidium spp.	13(8)	46(12)	45(14	18(12)	0 (0)	12(8)
Neozephlebia	8(3)	14(7)	0	0	0(0)	2(2)
Zephlebia	3(3)	12(8)	0	0	5(5)	18(8)
Megaleptoperla	88(56)	4(4)	0	0	8(3)	0
Aoteapsyche	301(296)	112(50)	1(0)	9(4)	3(3)	0
Hudsonema	77(77)	7(5)	0	0	19(3)	0
Hydrobiosis spp.	34(19)	0	3(2)	1(1)	16(5)	2(2)
Oxyethira	112(107)	0	0	0	0	0
Austrosimulium	13(3)	28(9)	0	4(2)	32(11)	66(34)
Chironominae	1171(771)	0	15(15)	0	3(3)	5(5)
Orthocladiinae	2827(256)	5(3)	6(5)	1(1)	184(29)	64(46)
Potamopyrgus	5944(5741)	432(272)	1(1)	4(3)	123(27)	84(81)
Elmidae	37(27)	2(2)	63(38)	8(4)	0	0

Viable cave populations

As previously mentioned aquatic communities within the caves were a subset of their upstream surface communities. If cave benthic communities were structured exclusively by drift the proportion of invertebrates drifting above the cave may relate to the proportion of invertebrates in the benthos within the cave. Taxa with low surface drift rates but high cave benthic densities may have sustainable cave populations, while taxa with high surface drift rates but low cave benthic densities may not be persisting within the cave, and taxa that had

surface drift rates appearing to be in ratio with cave benthic densities may have been solely structured by drift.

Table 2.2. Drift density of aquatic taxa (Ind. m^{-3} s^{-1}) at the surface (sites +128 m and +32 m averaged) and within the caves (sites -32 m, -128 m, -256 m averaged). Values in brackets are standard errors. Taxa shown were the most abundant. Mur = Murchies, Hh = Hollow Hill, Wi = Whites.

	Mur surface	Mur cave	Hh surface	Hh cave	Wi surface	Wi cave
Deleatidium spp.	0.0049 (0.0049)	0.1356 (0.0646)	0.010 (0.0077)	0.0185 (0.0078)	0	0.0068 (0.0045)
Neozephlebia	0.0008 (0.0008)	0.0116 (0.0071)	0	0	0	0.0026 (0.0015)
Zephlebia	0.0048 (0.0032)	0.0953 (0.0481)	0	0.0012 (0.0036)	0.0013 (0.0013)	0.0105 (0.0073)
Megaleptoperla	0.0016 (0.0016)	0.0031 (0.0016)	0	0.0004 (0.0009)	0	0
Aoteapsyche	0.0711 (0.0711)	0.0102 (0.0055)	0.0020 (0.0003)	0.0027 (0.0018)	0.0013 (0.0013)	0
Hudsonema	0.0057 (0.0057)	0.0062 (0.0006)	0.0024 (0.0024)	0.0027 (0.0018)	0.0026 (0.0026)	0.0009 (0.0005)
Hydrobiosis spp.	0.0178 (0.0064)	0.0098 (0.0049)	0.0114 (0.0098)	0.0187 (0.0058)	0.0082 (0.0031)	0.0048 (0.0027)
Oxyethira	0	0	0	0.0017 (0.0015)	0	0
Austrosimulium	0.0437 (0.0115)	0.0080 (0.0019)	0.0020 (0.0003)	0.0066 (0.0031)	0.0346 (0.0038)	0.0733 (0.0207)
Chironominae	0.0210 (0.0031)	0	0.0012 (0.0012)	0.0012 (0.0004)	0	0
Orthocladiinae	0.1098 (0.0270)	0.0024 (0.0015)	0.0193 (0.0184)	0.0137 (0.0014)	0.0173 (0.0083)	0.0236 (0.0202)
Potamopyrgus	0.4428 (0.4103)	0.0909 (0.0133)	0.0020 (0.0003)	0.0042 (0.0020)	0.0334 (0.0334)	0.0034 (0.0034)
Elmidae	0.0008 (0.0008)	0.0014 (0.0014)	0.0921 (0.0257)	0.0860 (0.0327)	0	0

Within Murchies cave, surface drift density (sites +128 m and +32 m, averaged) was significantly correlated to cave benthic density (sites -128 m and -256 m, averaged) (R^2 = 0.48, F = 11.12, df = 13, P < 0.001) (Figure 2.6). Several taxa (*Deleatidium* and *Neozephlebia*) had low surface drift rates but high cave benthic densities while other taxa (Orthocladiinae and Oligochaeta) had high surface drift rates but low cave benthic densities. The remaining taxa had surface drift rates that appeared to be in ratio with cave benthic densities.

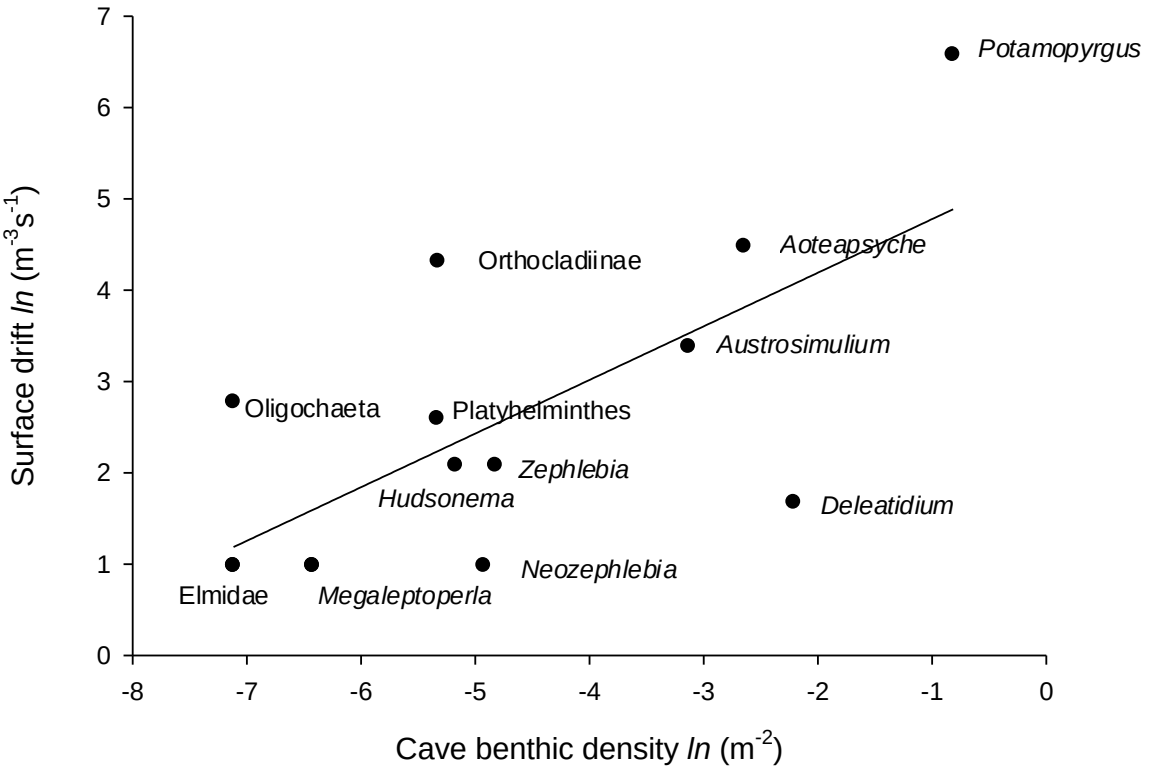

Figure 2.6. Murchies surface invertebrate drift density (+128 m and +32 m averaged) in relation to cave invertebrate benthic density (-128 m and -256 m averaged).

Within Hollow Hill cave, surface drift density (sites +128 m and +32 m, averaged) was not significantly correlated to cave benthic density (sites -128 m and -256 m, averaged) (R^2 = 0.60, F = 4.51, df= 4, *P* = 0.12), presumably because of *Aoteapsyche* (Figure 2.7). The net-spinning caddis *Aoteapsyche* had low surface drift rates but high cave benthic densities, while the other taxa had surface drift rates that appeared to be in ratio with cave benthic densities.

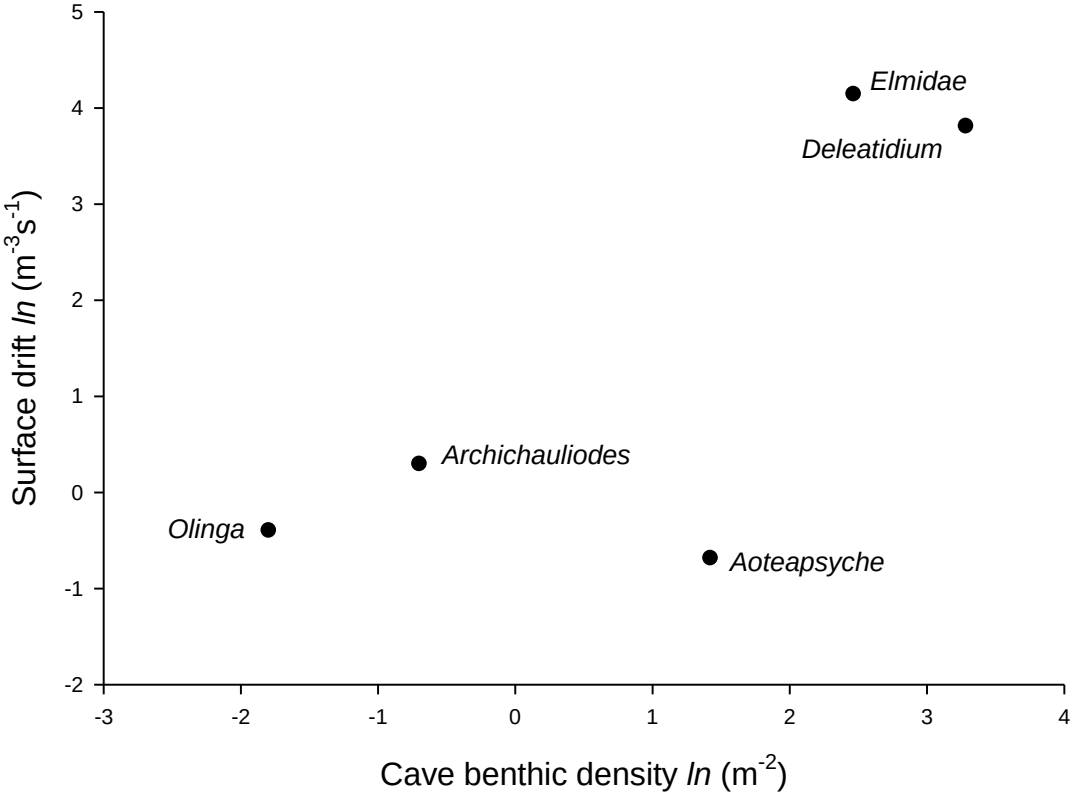

Figure 2.7. Hollow Hill surface invertebrate drift density (+128 m and +32 m averaged) in relation to cave invertebrate benthic density (-128 m and -256 m averaged).

Within Whites cave, surface drift density (sites +128 m and +32 m, averaged) was not significantly correlated to cave benthic density (sites -128 m and -256 m, averaged) (R^2 = 0.22, F = 2.01, df = 8, *P* = 0.20) (Figure 2.8). Z*ephlebia* had low surface drift rates but high cave benthic densities, whereas Tanypodinae, *Potamopyrgus*, Ostracoda, *Psilochorema*, and damselfly larvae (Zygoptera) had high surface drift rates but low cave benthic densities. The remaining taxa had surface drift rates that appeared to be in ratio with cave benthic densities.

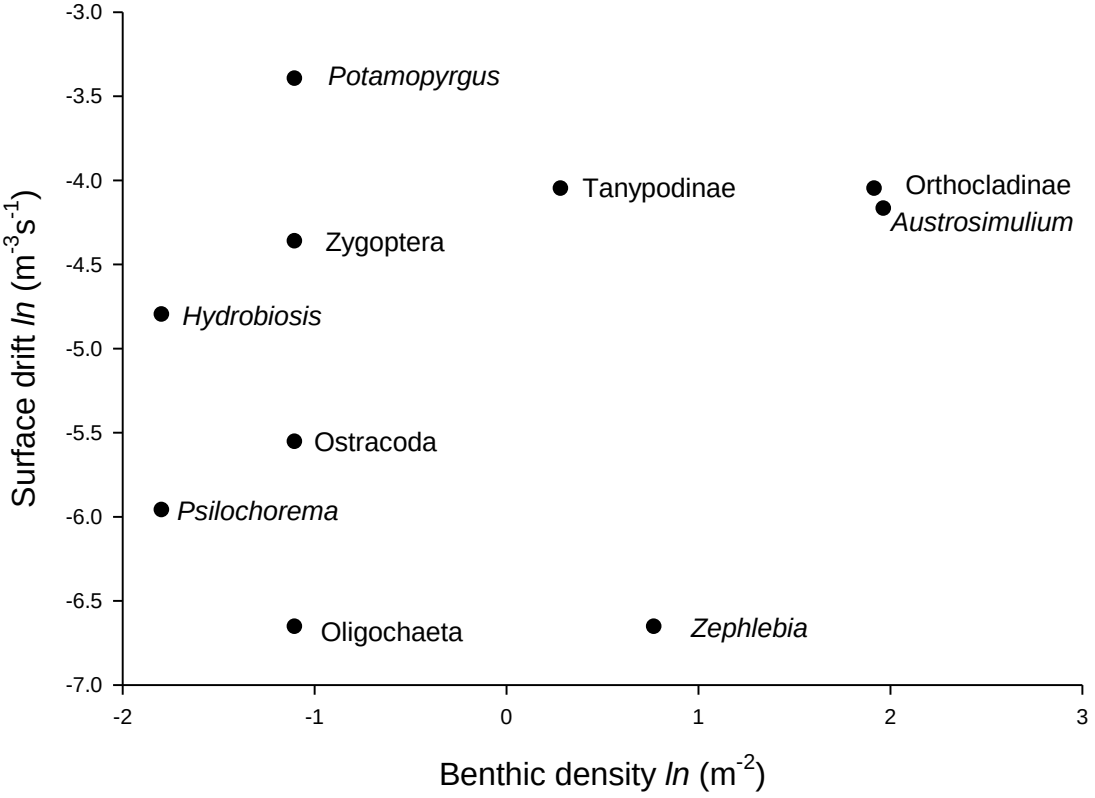

Figure 2.8. Whites surface invertebrate drift density (+128 m and +32 m averaged) in relation to cave invertebrate benthic density (-128 m and -256 m averaged).

Adult flight patterns

Numbers of adult insects captured on upstream and downstream sides of sticky traps were not significantly different ($P > 0.05$) so data were combined for analysis. Adult Ephemeroptera, Plecoptera, and Trichoptera (EPT) density on traps decreased rapidly within the caves (Figure 2.9). However, this was driven primarily by the agricultural streams, as EPT taxa were caught at only two forested sites over the sampling period. Significantly more EPT taxa were found outside caves than inside ($R^2 = 0.53$, $F = 11.41$, $df = 11$, $P = 0.007$).

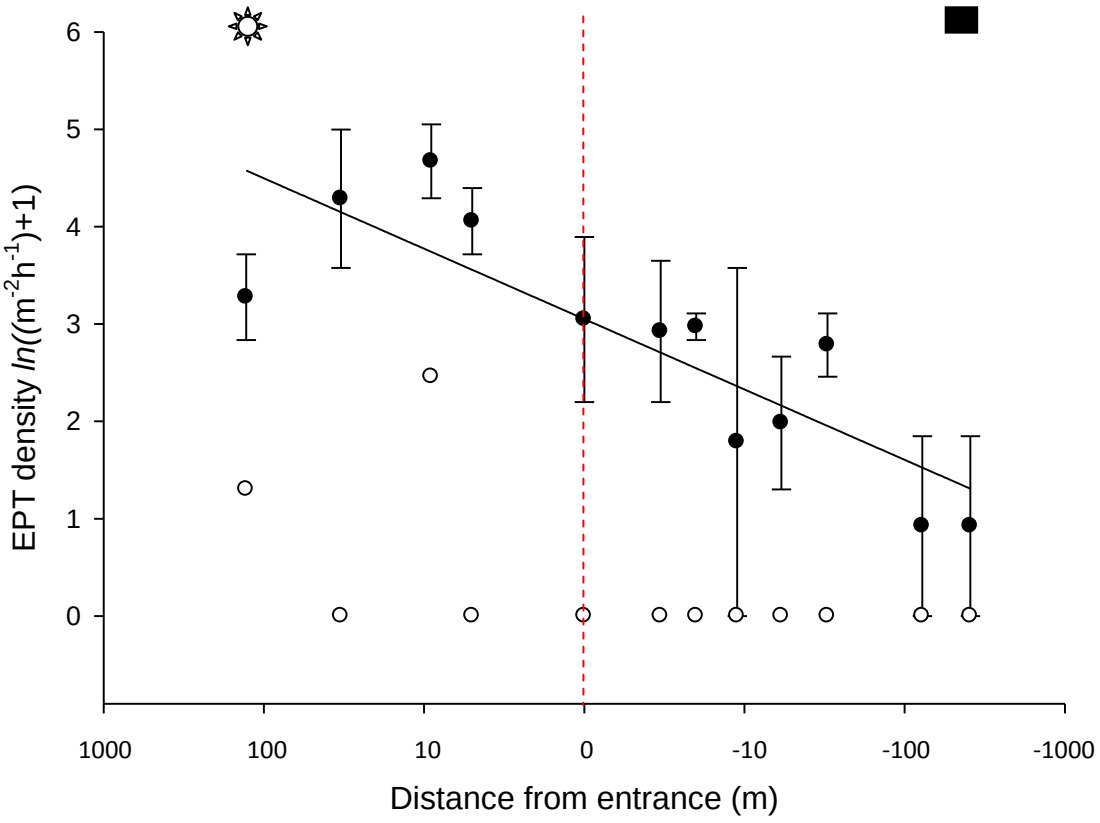

Figure 2.9. Mean density (± 1 standard error, n = 6) of EPT adults on sticky traps at the two pastoral streams (closed circles) and the forested system (open circles). Traps were left at each site for an average of 24 hours.

Community structure

Communities in the forested stream, Whites, contained different taxa and lower aquatic invertebrate densities, than the pastoral streams, Murchies and Hollow Hill. The pastoral stream communities had similar taxa, but density was higher in Murchies. In the forested stream, invertebrate communities in pools differed from those in riffle-runs (Figure 2.10). No single driver was significantly correlated with invertebrate communities across all caves. A CCA of the invertebrate communities in Murchies showed that water temperature, distance into cave, average wet width, and average depth were all significant drivers of community composition. A CCA of the invertebrate communities in Hollow Hill showed that wet width and chlorophyll-*a* were significant drivers of community composition. An RDA of the invertebrate communities in Whites showed distance from the cave entrance was the only

significant driver of community composition. All cave sites were generally closer together in ordination space than their associated surface communities.

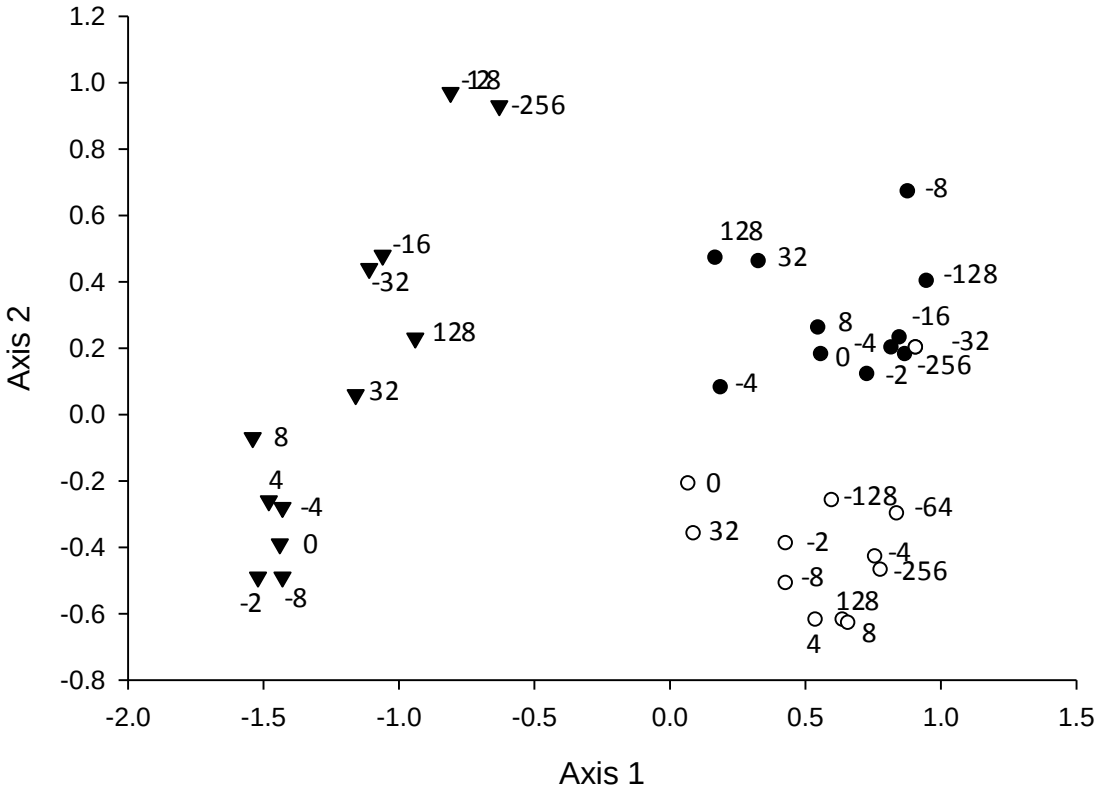

Figure 2.10. NMDS of aquatic invertebrate communities for all three stream systems. Numbers represent the distance from entrance, with negative numbers occurring inside caves. Inverted triangles represent communities in Whites, solid circles represent sites in Murchies, and open circles represent sites in Hollow Hill cave. Sites 8 to -8 in Whites were pool sites. The rest of the sites were in riffle/runs.

Discussion

Differences in cave and surface communities

Chlorophyll-*a* and CPOM biomass decreased rapidly at the cave entrances and were significantly lower within the caves, relative to surface environments, presumably because of the exclusion of light and riparian vegetation. However, despite the predicted importance of these factors they did not appear to be the main drivers of community structure. FPOM was observed on the streambed at all sites and likely played a significant role in structuring invertebrate communities. FPOM can be transported in the water column for long distances

(Ock 2010), and the lack of other kinds of food means that it potentially supplies a valuable resource, to cave stream communities. *Aoteapsyche* and *Deleatidium* were observed over one kilometre inside caves, and the presence of the filter-feeding *Aoteapsyche* suggests that FPOM is still a readily available resource. A study by Ock (2010) in dam tailwaters showed FPOM transport distances of up to 19.2 km in deeper channels (and a minimum transport distance of 2.2 km in shallower channels), so it is likely that invertebrate communities will have continual access to FPOM resources in cave systems. FPOM biomass may also remain high throughout caves as percolating water and entrances offer recharge points (Bolstad and Swank 1997).

As well as having an abundance of carbon resources, surface streams in this study had a diverse range of micro-habitats, allowing greater taxa diversity and density (Gray et al. 2006). Surface sites ranged from shallow algal riffles, to deep macrophyte filled runs, to debris-shrouded pools, whereas caves generally consisted of bedrock riffles, bedrock runs, and bedrock pools. The loss of micro- and macro-habitats combined with a reduction in energy supplies (food and solar), are likely to have had cumulative negative effects upon cave stream communities.

Invertebrate benthic richness and density were significantly lower within caves than in the surface streams. Furthermore, cave invertebrate communities were found to be a subset of surface communities, sourced through downstream drift, as also found by Death (1989). Taxonomic diversity was closely associated with invertebrate density, which was not surprising as lower density communities are likely to contain fewer taxa and rare species, as seen in other studies (e.g. Collier and Quinn, 2003). The decline in taxonomic richness and density within caves was presumably related to a loss of suitable resources (Death 1989). However, within caves benthic invertebrate density and richness did not vary between -32 and -256 metres (across all sampled caves). This finding suggests that conditions were relatively constant within caves enabling similar invertebrate richness and densities, even where land-use differed upstream. The significant increase in drift propensity, inside caves, suggested an increase in relative densities of organisms entering the drift, potentially because of a lack of suitable resources, i.e. caves are resource limited, rather than structured by increased predation levels (Peckarsky 1983). It is also possible that the lack of light was responsible for increased drift propensity within caves, as it is known that most invertebrates have a diel drift pattern, with a peak at night (Elliott 1968, McIntosh 2000, Duan et al. 2008).

The constant invertebrate drift density observed between the inside and outside of caves was presumably because of invertebrates attempting to leave an unsuitable environment.

Significantly fewer adult EPT taxa were recorded within caves, possibly because of the lower number of juveniles within them, or because few adults fly into caves. The low numbers could also be partially explained by lower temperatures that may limit flight (Smith 2002), and some adults may be attracted and ensnared by the glow-worm *Arachnocampa luminosa*.

The larvae of individual aquatic taxa differed in their "responses" to cave environments, and surprisingly, mayflies and *Austrosimulium* were found in greater numbers in the benthos within caves than outside them. The larger numbers in caves could be related to an increase in relative resource availability, because of the decrease in total invertebrate density, or a decrease in predatory invertebrates, primarily *Hydrobiosis* species, and possibly *Hudsonema* species, as they are known omnivores (Winterbourn 2000). *Oxyethira* was presumably not found on the benthos within caves because filamentous algae, on which it feeds (Quinn and Hickey 1990), were only present outside the caves. Predatory invertebrates (primarily *Hydrobiosis* spp.) were presumably not found within the caves because of the decrease in total prey density, or because harsh abiotic conditions (a preponderance of bedrock lacking refuges for predators) make predation difficult (Peckarsky 1983). Elmidae also appeared to find the cave environment unsuitable, and had low benthic density but increased drift density. Chironomids too were rarely present within the caves. Most of those outside the cave were tube-dwelling species and unlikely to drift, and it appears that few adults were attracted into the caves to lay eggs. Death (1989) obtained similar results, except that mayflies were less abundant in the cave he studied.

Source-sink dynamics

Source-sink dynamics in caves would seem to be driven by drift, either active or passive. I found that high surface drift densities did not necessarily equate to high cave benthic densities, suggesting that the presence of a cave altered populations. However, because drift was only sampled once, conclusions drawn from the results are necessarily tentative. My data showed that in the Murchies system Oligochaeta and Orthocladiinae were present at lower densities than drift predicted, whereas in Whites, Tanypodinae, *Potamopyrgus,* Ostracoda, *Psilochorema*, and Zygoptera were present at lower densities than drift predicted. It is therefore possible to surmise that these particular species were unable to maintain populations

in the cave and were likely experiencing a mortality level greater than the drift rate, or an emigration rate higher than an immigration rate. Conversely, *Deleatidium* and *Neozephlebia* were present in greater density than drift predicted in Murchies, while in Hollow Hill and Whites *Aoteapsyche* and *Zephlebia*, respectively, were both present at higher densities than drift predicted. It is therefore possible that these particular species were able to maintain populations greater than drift predicted, either through organisms reproducing within the cave and completing their life cycles, or because the mortality rate was lower than the rate of replacement, via drift. As to which of these hypotheses was correct cannot be confirmed by my data, but I would speculate that for *Zephlebia* hypothesis two is likely to be correct. While it may be possible that the net spinning caddis *Aoteapsyche* is able to attract mates in darkness, with the aid of pheromones, it seems unlikely, and further research is needed.

Forested and pastoral aquatic communities

An NMDS performed on benthic data from the three caves combined showed they all had significantly different communities, although the two pastoral streams had similar species. This result was not surprising as large and small differences between each catchment result in differences in community structure. The fact that species composition was similar between the two pastoral streams and very different from the forested stream is neither surprising nor novel (Quinn and Cooper 1997, Young and Huryn 1999, Collier and Quinn 2003, Death et al. 2003). Community densities were highest in surface pastoral sites, followed by surface forested sites, and lastly within caves. Beyond the twilight zone, community density was lowest, with no apparent difference between caves in catchments supporting different land uses. Adult EPT flight was greater in the pastoral systems, possibly because of higher larval densities, solar radiation, and temperature.

An important factor structuring cave invertebrate communities is the ability of species to colonise them. If reproduction is not occurring within caves, organisms must be entering them from an outside source population, driving a source-sink relationship. There is no published evidence of surface aquatic macroinvertebrates completing their life cycle within caves, and I found none in the caves I studied. It is also unlikely that individuals from deeper cave sites fly out of the caves to reproduce, especially in the forested system where no individuals were recorded flying within the cave.

Within my study, differences occurred between invertebrate communities within forested, pastoral, and cave streams thought to be related to carbon resources. However, it is possible that observed differences were not only because of food availability, but other environmental drivers. An alternative hypothesis is that differences between forested, pastoral, and cave stream communities were driven by recruitment rate not simply a lack of available food.

High light and temperatures in surface streams can be expected to lead to increased larval metabolism (Biswas 1991, Hervant et al. 1997, Hervant and Renault 2002, Issartel et al. 2005), and adult flight activity (Smith 2002), enabling growth and reproduction to occur at a faster rate, relative to cave invertebrates. This in turn could lead to denser populations in warm, light-abundant systems, such as pastoral streams. Adult flight would therefore be expected to be greater in open and warmer sites (e.g. Winterbourn and Crowe, 2001; Collier and Quinn. 2003), and benthic populations would be dominated by tolerant organisms with high reproduction rates, such as *Deleatidium* (Winterbourn and Crowe 2001). Therefore caves, devoid of light, would be expected to support lower invertebrate growth and maturity (Kinsey et al. 2007), as well as limited adult flight and reproduction, leading to depauperate communities sustained only by an upstream source.

In conclusion, the two surface pastoral streams had similar species richness and greater invertebrate densities than the forested stream up-stream of the cave entrance. However, 32 meters inside the caves species density and richness were similar, suggesting environmental conditions were similar. No single physico-chemical driver of community assemblage was significant across all three caves. More importantly, the measured food resources (algae and CPOM) did not appear to structure aquatic communities. Coarse particulate organic matter (CPOM) was not a significant driver of community assemblage in any of the streams, whereas filamentous algae were only important in one pastoral stream, despite both surface streams containing them. Finally, cave streams had lower invertebrate richness and density. Cave aquatic communities appeared to be structured by a lack of resources, leading to increased drift propensity. Furthermore, cave communities appeared to be a sink derived from the upstream surface source community. However, some taxa were found in greater numbers than drift predicted, suggesting that an organism's physical and behavioural adaptations, as well as resource/environmental gradients, were important for within-cave survival. Additional, more intensive, long-term studies are clearly needed to further assess the major drivers of cave community structure.

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Ameletopsis (a predatory mayfly) 200 m within a cave

Chapter 3

Factors limiting macroinvertebrate communities within cave streams: manipulations of algae and CPOM

Abstract

Pastoral and forested surface streams contain markedly different invertebrate communities, primarily because of their differing dominant basal resources: algal versus CPOM. These characteristics should also remain apparent within recipient cave streams. I manipulated CPOM and algal abundance within streams to investigate their relative importance to forested and pastoral aquatic invertebrate communities. Pre-conditioned replicate algal tiles were placed outside and inside two caves. Tiles either excluded (control) or were exposed to invertebrate grazing, and left for five days. In a second manipulation, CPOM (leaf packs) and an inedible artificial habitat (plastic leaves) were added across the cave ecotone, and left for 41 days. Unfortunately, a bacterial bloom in the forested stream smothered benthic communities making it impossible to compare results. However, algal biomass declined within the cave on the pastoral stream, although there was no significant difference between biomass on exclusion and open tiles. Thus, algae may have died in the cave within 5 days due to a lack of light, and grazing had little effect; or algae were consumed rapidly by invertebrates on open tiles, while decreasing gradually on grazer-exclusion tiles to a level similar to that of open tiles, over the duration of the study. Invertebrate abundance on leaf packs or artificial packs, showed no significant difference inside and outside the cave, suggesting that caves were resource limited. Furthermore, leaf and artificial packs appeared to be colonised by individuals in the drift, rather than from the benthos. Leaf packs had significantly more taxa and denser communities than artificial packs, suggesting that actual leaves were the preferred resource. Shredders were the only functional group that increased in abundance in leaf packs, relative to control or survey data. Finally, aquatic invertebrate communities inside the cave were a subset of surface communities and appeared to be resource limited.

Introduction

Energy driving stream ecosystems comes either from in-stream photosynthesis (i.e. autochthonous sources), or allochthonous materials (Hicks 1997, Huxel and McCann 1998). Autochthonous production (i.e. algal growth), occurs wherever light is present, although environmental factors, such as floods or droughts, can lead to reductions in primary production (Whiles and Wallace 1995, Townsend et al. 1997, Collier and Quinn 2003). Whereas variations in light energy affect photosynthesis directly, they also affect growth indirectly, as solar radiation controls temperature, which in turn influences primary production (Azad and Borchardt 1969). A third factor having a positive effect on photosynthetic production is nutrient addition, particularly concentrations and availability of nitrate and phosphate (McDowell et al. 2009). Differences in algal density, between forested and pastoral streams, are therefore primarily due to variations in light, temperature, and nutrient levels (Winterbourn and Fegley 1989, Niyogi et al. 2007b). It has been suggested that primary production is positively correlated with food chain length, or ecosystem complexity, such that streams with higher levels of primary production (e.g. pastoral streams) will have longer food chains and increasingly complex ecosystems, compared to streams with lower levels of primary production, such as forested streams or streams in caves (Huxel and McCann 1998, Simon et al. 2003).

Terrestrially derived, allochthonous, organic matter has been shown to be important in many stream ecosystems and its presence and abundance are primarily dependent upon the form and structure of riparian vegetation (Fisher and Likens 1973, Cummins 1974). Detritus, leaves and wood from the riparian zone, and dead or decaying material produced within the stream are transformed by abiotic and biotic processes while undergoing displacement downstream, and provide important basal resources for stream communities (Suberkropp and Klug 1980, Wallace et al. 1982, Boulton and Boon 1991, Suberkropp and Chauvet 1995, Young et al. 2008). Leaf breakdown typically occurs in three phases (against a background of physical abrasion); 1) a rapid loss of mass due to leaching of soluble compounds, 2) colonisation by microorganisms, 3) losses from invertebrate shredding (Burton and Likens 1973, Quinn and Cooper 1997, Rutherford et al. 1997). As a result, streams have strong linkages to both lateral and upstream terrestrial components of the landscape. These linkages are particularly important to cave stream ecosystems because of their reliance upon detrital

material, and an absence of autochthony (Culver 1982, Simon and Benfield 2001, Simon et al. 2003).

Land use change

A reliance upon surface-derived material suggests that structuring of macroinvertebrate communities within caves should be strongly linked to land-use practices upstream (Sponseller et al. 2001). A loss of terrestrial vegetation can lead to alterations in flow regimes (Poff et al. 1997), evapotranspiration, sedimentation, channel morphology, solar radiation (Burton and Likens 1973, Quinn and Cooper 1997, Rutherford et al. 1997), temperature (Vannote and Sweeney 1980, Ward and Stanford 1982, Quinn and Hickey 1990, Quinn et al. 1994, Rutherford et al. 1997), and organic matter inputs, which can bring about changes to surface (Lenat and Crawford 1994, Waters 1995, Quinn and Cooper 1997, Sponseller et al. 2001) and cave communities (Simon et al. 2003, Wood et al. 2008).

In New Zealand, land-use change affects water quality, physical habitat and the nature of the biota within streams (Quinn and Cooper 1997, Harding et al. 1999). The conversion of shaded forest streams to open waterways alters stream energy sources, may permit grazing to channel edges by stock, and often results in bank erosion, higher water temperatures, and higher nutrient inputs (Williamson et al. 1992, Quinn 2000). Loss of riparian vegetation reduces the amount of wood and leaf litter entering streams (Scarsbrook et al. 2001) and destabilises banks, previously bound by tree roots (Hanchet 1990). Deforestation has also led to increased peak flows and water volumes during flood events (Rowe et al., 1997). Collectively, these impacts constitute a press disturbance and can continue to affect stream community structure and function in the long term (Harding et al. 1998).

Resource additions

Many organisms live in or on their food and in such cases it may be difficult to assess the relative importance of material as habitat or food (Dobson 1991). In an attempt to distinguish between habitat and food limitation, several studies have compared colonisation of real and artificial leaves by invertebrates (Dobson and Hildrew 1992, Richardson 1992b, 1992a, Wallace et al. 1999). In most instances, invertebrate diversity and density has been found to be higher on natural leaves than artificial ones (Dobson and Hildrew 1992, Richardson 1992b, 1992a, Wallace et al. 1999). For example, Richardson (1992b) found that food, not

habitat, was the primary determinant of leaf pack use for most invertebrates (regardless of their feeding strategy) in a North American stream, and that shredders increased in numbers on leaf packs, relative to controls. Nevertheless, it is likely that organisms also use leaves to shelter from currents, for settlement, refuge from predators, and to shelter between foraging forays (Richardson 1992b). Wallace et al. (1997) excluded leaf litter inputs to a forested stream reach and demonstrated the powerful influence of allochthonous inputs in structuring stream food-webs. Cave streams provide a natural situation that is the reverse of the Wallace et al. experiment as allochthonous inputs enter them in the drift, while autochthonous production ceases (Simon and Benfield 2001).

The aim of this study was to test the importance of algal and CPOM resources to aquatic macroinvertebrate communities within cave streams, relative to forest and pastoral systems. I predicted that algae would be lost at a faster rate within a cave on a pastoral stream because of the lack of algal resources within caves. I also predicted that macroinvertebrates would be found in greater densities on leaf packs relative to artificial packs, because of their higher nutritional value. Conversely, I predicted that algae would be lost in a forested stream, at a similar rate inside and outside the cave, because of similarly low light levels both above and within the cave. As a result, macroinvertebrates would be found at greater densities on leaf packs relative to artificial packs, because of their higher nutritional value, and a shift in density would occur between the inside and outside of the cave, because of the greater abundance of CPOM outside the cave.

Methods

Study streams

The study was conducted in two streams (Murchies and Whites) located in the Waitomo region, central North Island, New Zealand. Waitomo is predominantly hilly, with sheep and beef farming common and with fragments of native forest, comprising podocarps, ferns, and hardwood. Geologically the area is dominated by karst (Williams 1992).

Murchies Stream is located 7.8 km from the town of Waitomo and flows into a limestone cave approximately 1 km long (1785080 E, 5767710 N). The catchment is approximately 1.5 km^2 and predominantly covered in native podocarp forest. The stream is second order and flows through approximately 0.95 km of sheep and beef farmland before entering the cave.

Murchies cave is characterised by a series of riffles, runs and pools overlying limestone bedrock substrate.

Whites Stream is 17.3 km from the town of Waitomo and has a limestone cave approximately 1.1 km long (1.773340 E, 5762940 N). The catchment area is approximately 1.5 km^2, predominantly covered by native podocarps. The stream has two tributaries, one of which is sourced from a farm pond and wetland and one, the larger, from native forest. The stream is composed of riffles, runs, and pools with a limestone bed and silt filled pools.

Algal manipulation

Algae were allowed to colonise 144 ceramic tiles (24.95 cm^2) in an open section of stream, between 14 December 2009 and 14 January 2010. Tiles were placed at +128 m, +64 m, +32 m, -32 m, -64 m, and -128 m, in the forested and pastoral streams (where positive values are sites outside of the cave, 0 m is the cave entrance, and negative values are sites inside the cave). At each site ten tiles were deployed, five with grazer exclusion cages and five without grazer exclusion cages (= open). Grazer exclusion cages consisted of sections of corrugated pipe (150 mm diameter) cut longitudinally to form a trough and covered with 1 mm mesh (Figure 3.1). Tiles were placed at their respective sites on 14 January and removed five days later when they were frozen for transportation to the laboratory.

Figure 3.1. A grazer exclusion (left), and open treatment (right) in the pastoral stream.

In the laboratory, tiles were scrubbed with a toothbrush and filtered onto pre-ashed glass microfibre filters (diameter 47 mm, nominal pore size 1 µm), dried for a minimum of 72 h at 50°C, weighed and ashed at 500°C for 3 h and re-weighed to calculate ash free dry mass (AFDM). Initial algal masses were assumed from single randomly removed control tiles taken from each set of six that had been placed together for initial colonisation and had visually similar algal densities.

Leaf pack manipulations

All leaf packs were made from onion bags containing either 5 g of dried willow leaves (*Salix* sp.) or 5 g of uncut flagging tape. Three leaf packs and three artificial leaf packs were placed at +128 m, +32 m, +8 m, +4 m, 0 m, -2 m, -4 m, -8 m, -16 m, -32 m, -128 m, and -256 m, in the forested and pastoral streams. Packs were left in the streams for 41 days. Between 1 and 2 March 2010 all packs were removed and frozen. In the laboratory leaf packs were washed on a 250 µm mesh sieve, and invertebrates were removed and identified using a 40x Wild M3 dissecting microscope. Identifications were made using the keys and guides of Winterbourn (1973) and Winterbourn et al. (2000). Identification was carried out to the lowest taxonomic level possible, except for Chironomidae, which were not separated below tribe. Remaining material was placed in pre-ashed weigh boats and dried for a minimum of 72 h, weighed and ashed for 3 h at 500°C, before re-weighing, to calculate AFDM.

Invertebrates were assigned to functional feeding groups using Winterbourn (2000), Quinn and Hickey (1990), and personal communications with Emeritus Professor Mike Winterbourn.

Algal and leaf pack manipulations were performed in and upstream of both pasture (Murchies Stream) and forest (Whites Stream) caves in order to compare land-use effects. Unfortunately, in the forested system, a bacterial bloom, generated in an upstream swamp, smothered the streambed, algal tiles and leaf packs, leading to a near complete loss of invertebrates; only three aquatic individuals were found and there was an absence of spiders in riparian vegetation. Interestingly, at the end of my field season a flushing flow removed the bloom, and aquatic invertebrates and riparian spiders reappeared. However, results obtained from the forest stream were not able to be compared with those from the pasture stream, and are not considered further.

Statistical analysis

In order to test for differences in algal abundance in the presence and absence of grazing invertebrates, both inside and outside the caves, I used two way analysis of variance (ANOVA). Factors were transformed, ln(x + 1), to meet assumptions of normality and homogeneity of variances (Quinn and Keough 2002).

To assess differences in the percentages of functional feeding groups between leaf packs, artificial packs, and 2009 survey data I used Generalised Linear Models (GLMs). GLMs were used because distance from entrance was a continuous variable. The response percentage of each functional feeding group was arcsine square-root transformed, to meet assumptions of normality and homogeneity of variances (Quinn and Keough 2002).

Non-metric multidimensional scaling (NMDS) ordinations were used to investigate macroinvertebrate community composition in leaf packs and artificial packs inside and outside the cave. Site 0 m was classified as outside the cave because of the presence of light. Macroinvertebrate abundance data were log (x + 1) transformed to down weight the contribution of rare species. The NMDS ordination, used the Bray-Curtis distance measure, so that distance between sites reflected degree of similarity in taxonomic composition (Jongman et al. 1995, Quinn and Keough 2002).

One-way analysis of similarity (ANOSIM) was performed on a dissimilarity matrix using the Bray-Curtis distance measure to test whether there were significant differences between leaf and artificial packs inside and outside the cave. Calculations were made in Primer 6.1.12 (Clarke and Warwick 2001). The significance score indicates variability, a score of 0 indicates no difference between groups, 1 indicates complete difference, and a negative value indicates very high variability (Quinn and Keough 2002). A *P*-value was calculated for each interaction between the four factors.

Similarity percentages (SIMPER) were calculated for all interactions between leaf and artificial packs inside and outside the cave. SIMPER indicates which species are responsible for observed patterns in the non-metric multidimensional scaling plot (NMDS) by calculating average Bray-Curtis dissimilarity between all pairs of inter-group samples (Clarke 1993). Analyses were again performed in Primer 6.1.12 (Clarke and Warwick 2001).

Results

Algal manipulation

In the pastoral stream algal growth occurred outside the cave on both exclusion and open tiles, whereas algae were completely lost within the cave on both exclusion and open tiles (Figure 3.1). Significantly more algae were lost within the cave (Two way ANOVA, F = 19.26, df =12, $P = 0.002$), but no significant difference was observed between tiles open to or excluded from grazers ($P > 0.05$).

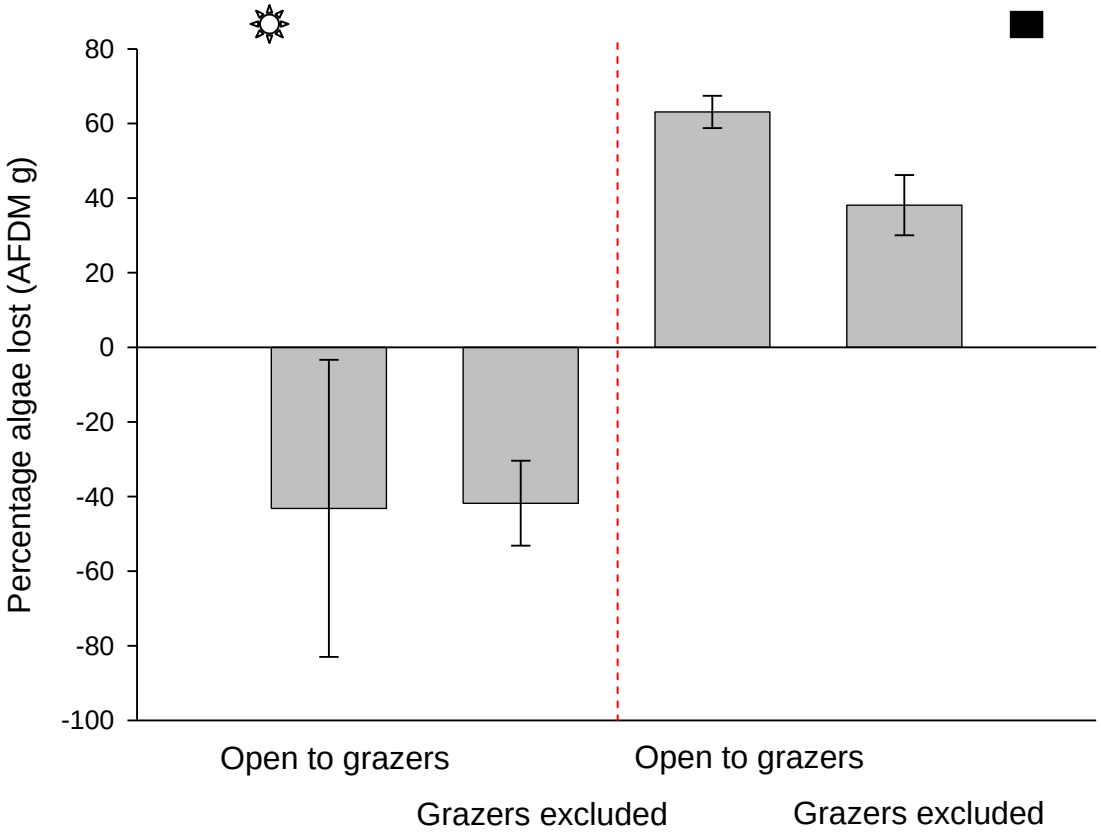

Figure 3.1. Percentage of algal biomass lost inside and outside a cave in treatments open to grazers and from which grazers were excluded. Mean ±1 standard error (n = 6). In this figure and the following (unless otherwise stated) the dotted line represents the cave entrance, the sun represents outside the cave, and the black square represents inside the cave.

Leaf pack additions

A GLM showed significantly more taxa on leaf packs than artificial packs (df = 66, P = 0.018). However, there was no significant difference between species richness and distance (df = 66, P = 0.22), and no significant interaction between distance and treatment (df = 66, P = 0.49) (Figure 3.2). A second GLM showed significantly more abundant communities in leaf packs than in artificial packs (df = 66, P = 0.017), but again there was no significant difference between species richness and distance (df = 66, P = 0.20), and no significant interaction (df = 66, P = 0.83) (Figure 3.2). There was no significant difference (P > 0.05) in the average dry weight of leaves lost per site (mean 1.6 g ± SE 0.2). Data for site +128 m were removed from these models as the treatments were buried by silt.

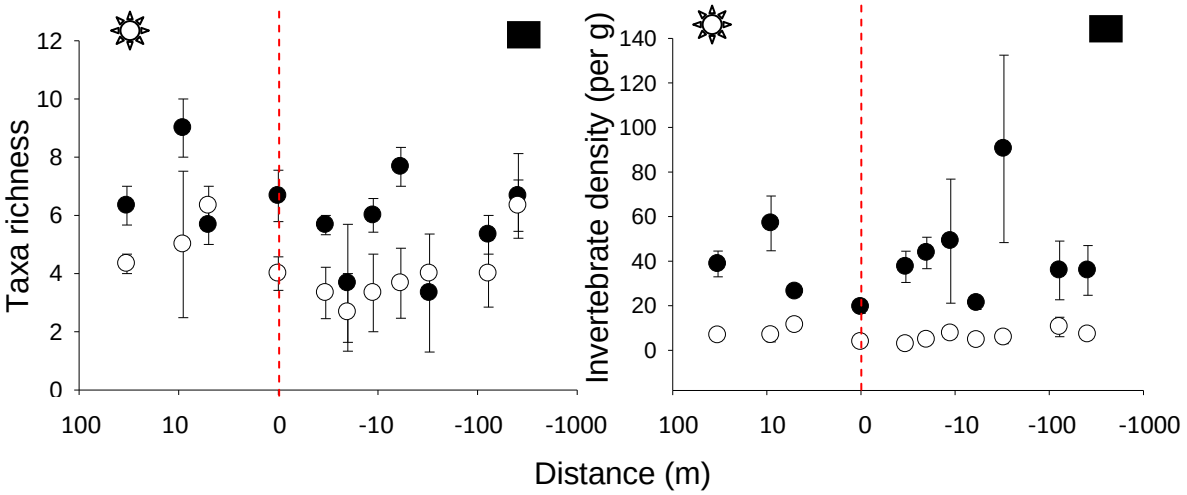

Figure 3.2. Macroinvertebrate taxonomic richness and density in leaf packs and artificial packs at 11 sites from +32 m upstream of the cave to -256 m within the cave. Mean ±1 standard error (n = 3). Solid circles represent leaf packs, and open circles represent artificial packs. Where error bars are not shown they were smaller than the symbol.

Results of GLMs used to test for significant differences in functional feeding groups (FFGs), among leaf packs, artificial packs, 2009 survey data, and distance from entrance are shown in Table 3.1. Significant differences between factors, for each FFG except algal piercers, were observed. Distance from the entrance was not a significant predictor of community

composition, while the interaction of treatments and distance was only significant for predators.

An NMDS and an ANOSIM of invertebrate abundance showed (after outliers -8 and +128 where removed, as these sites were buried in silt or washed on to the bank) significant differences in community composition between all leaf packs and artificial packs, and between leaf packs within and outside the cave (Figure 3.3). Artificial packs did not vary in community composition inside or outside the cave. A SIMPER analysis showed which species were driving differences between groups (Table 3.2). Significant differences were found between all treatments, except between controls outside the cave and controls within the cave (for more details refer to Table 3.2).

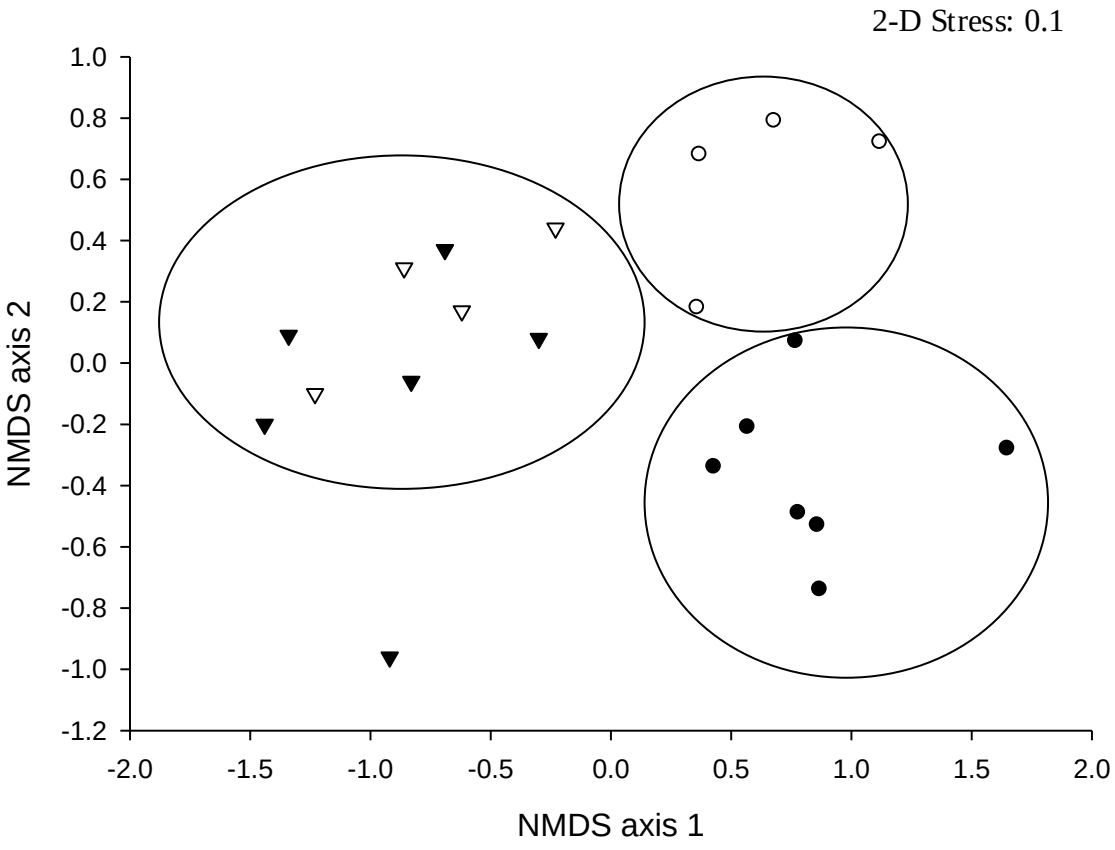

Figure 3.3. Non-metric multidimensional scaling ordination, of invertebrate communities in leaf packs within the cave (solid circles, n = 7), leaf packs outside the cave (open circles, n = 4), artificial packs within the cave (closed triangles, n = 7), and artificial packs outside the cave (open triangles, n = 4).

Table 3.1. Results of generalised linear models (GLMs) comparing relative abundances of functional feeding groups between leaf packs (L), artificial packs (A), and 2009 Surber sample data (S). Significant values are in bold.

	Factor	Significance	Distance	Factor* Distance
Collector/ Browsers	**df=96, f=11.17, *P*<0.01**	**L=A<S=L**	df=96, f=0.01, *P*=0.92	df=96, f=1.87, *P*=0.16
Filter Feeders	**df=96, f=7.96, *P*<0.01**	**L=A<S**	df=96, f=0.06, *P*=0.81	df=96, f=0.74, *P*=0.48
Algal Piercer	df=96, f=1.26, *P*=0.29	L=A=S	df=96, f=2.84, *P*=0.10	df=96, f=0.37, *P*=0.69
Predator	**df=96, f=12.59, *P*<0.01**	**A=L>S=A**	df=96, f=0.26, *P*=0.61	**df=96, f=4.99, *P*=0.01**
Scraper	**df=96, f=6.33, *P*<0.01**	**L=S>A**	df=96, f=1.45, *P*=0.23	df=96, f=0.28, *P*=0.76
Shredder	**df=96, f=13.26, *P*<0.01**	**L>A=S**	df=96, f=2.85, *P*=0.09	df=96, f=2.17., *P*=0.12

Table 3.2. Pairwise SIMPER comparisons of community dissimilarity among aquatic macroinvertebrate taxa, between leaf and artificial packs inside and outside the cave. Average percentage dissimilarity (Av. diss.) of community composition among leaf and artificial packs are shown, along with total abundances and percentage contributions of taxa that explained > 50% of the community.

Species	**Total abundance (invertebrates per g of leaves)**		**% contribution**
	Leaf packs outside cave	Leaf packs in cave	Av. diss. 45.7%
Neozephlebia scita	0.2	1.8	18.4
Aoteapsyche spp.	1.4	0.7	12.3
Deleatidium spp.	0.9	0.1	9.7
Potamopyrgus spp.	3.1	3.4	7.2
Hydrobiosis spp.	0.9	0.6	6.9
	Artificial packs outside cave	Artificial packs in cave	Av. diss. 35.6%
Aoteapsyche spp.	0.6	0.3	22.6
Potamopyrgus spp.	1.7	1.5	21.1
Orthocladiinae spp.	0.3	0.4	13.6

Discussion

Algae

The total amount of algae found inside and outside the cave differed significantly presumably because of the loss of light, as found in other studies (Azad and Borchardt 1969, Issartel et al. 2005). The similarity between grazing and exclusion treatments indicated that grazing alone did not explain reductions in algal biomass. Algal biomass at sites outside the cave, increased for two possible reasons, 1) algae were not used by invertebrates as a resource, or 2) algal biomass was not controlled by grazing pressure. However, algal biomass was also greatly reduced within the cave, such that there was no difference between grazed and non-grazed tiles. This was either a result of 1) algae dying due to lack of light, 2) algae being consumed rapidly by invertebrates, or 3) a combination of the two processes. I had expected that algae would be lost faster on open tiles within the cave, due to the lack of light. However, because I was not able to sample tiles during the five day experiment it was not possible to assess rates of change, and therefore my hypothesis was unable to be tested. An improvement to this experiment would be to have greater replication and to remove algal tiles over time, allowing the rate of change to be assessed more directly.

CPOM

Chapter two showed a decrease in invertebrate abundance and richness within the caves (Figure 2.4). However, in the CPOM addition experiment there was no significant difference between the density of invertebrates (per gram of leaves) inside and outside the cave. This suggested that communities within the cave were resource limited, and the addition of resources allowed aquatic invertebrate communities to persist within the cave environment. Significant changes in community composition were observed between leaf and artificial habitat packs; more taxa and greater densities of macroinvertebrates were found in leaf packs. This finding suggested that leaves (and their associated biofilms) may represent a limiting resource, and were inhabited for their nutritional value over and above their habitat value, as found in previous studies (Boutin 1990, Hart and Robinson 1990, Richardson 1991). Total grams of leaf lost per leaf pack did not differ significantly, inside and outside the cave. This result was probably because of mechanical losses and leaching, the fact that shredder density did not vary inside and outside the cave, or a combination of the two factors.

Colonisation

Invertebrates potentially colonised leaf packs in two ways: 1) by drift, or 2) by active movement on the stream bed. The majority of stream invertebrates are separated in space and time from their resources, i.e. decoupled (Hart and Finelli 1999). Therefore, it is unlikely that individuals were being attracted to the leaf packs by an environmental cue. The constant density of individuals in the drift, inside and outside the cave, meant constant densities of individuals were drifting, at any given site (Figure 2.5). It is therefore more likely that colonisation of both leaf packs and artificial packs was influenced by the density of individuals in the drift rather than their numbers on the bottom. However, the causes of invertebrate drift may differ inside and outside the cave (Death 1989), possibly due to competition outside and resource limitation inside (Peckarsky 1983). Nevertheless, caves would appear *prima facie* to be resource limited, relative to surface systems.

Invertebrate community composition on leaf packs varied slightly between surface and cave sites suggesting an individual's behavioural and physical adaptations may have been related to its survival. The difference between communities on leaf packs inside and outside the cave was driven primarily by *Neozephlebia*, which was 941% more abundant within the cave. This was presumably because *Neozephlebia* was more abundant within caves within the 2009 survey (Table 2.1), and the addition of a food resource further increased the density of *Neozephlebia* within the cave. This may have been due to a superior foraging technique allowing *Neozephlebia* to locate leaf packs and persist in the cave environment, or simply a decline in cave intolerant populations, and thus a reduction in competition.

Functional feeding groups

Comparison of functional feeding groups in leaf packs and artificial packs, and with the 2009 benthos data enabled the relative importance of resources and/or habitat to be assessed with respect to the benthos. Collector browsers were significantly under-represented on artificial packs potentially due to low food availability in this un-conditioned habitat. Filter feeding taxa (primarily *Aoteapsyche*) were dominant on the benthos potentially due to the unsuitability of leaf and control packs as habitat, for net spinning, possibly because of an absence of more-or-less laminar flow (Holomuzki et al. 2010).

Oxyethira was the only algal piercer found during my study and was mainly found outside the cave, presumably because filamentous algae on which it feeds (Quinn and Hickey 1990)

were only present outside the cave. Drift would have carried these organisms into the cave, but because of their inability to feed on detritus or epilithic biofilms they were rarely found within the pastoral cave. The lowest percentage of scrapers was found on artificial packs probably due to a lack of suitable food.

Predators were dominant on leaf packs possibly because CPOM provides a food and habitat resource, a higher prey density, or leaf packs comprise a habitat with resident predators and others that visit to forage (Andren 1992, Prous et al. 2004). The survey data, comprising animals from multiple habitats, had the lowest percentage of predators, and intermediate numbers were found on artificial packs where prey was less abundant than on leaves. Predators (primarily *Hydrobiosis* spp. and Tanypodinae) were significantly more abundant at sites deep within the cave, potentially because of increased foraging on introduced substrata within a food limited system.

Shredders were found in significantly higher percentages on leaf packs as they feed directly on the leaves (Winterbourn 2000). The inclusion of benthic data suggests that the pastoral stream was especially CPOM limited, with respect to shredders. This was not surprising as pastoral streams generally have low CPOM inputs (Townsend et al. 2004). However, shredders (primarily *Austroperla*) made up a low level of total density.

Further research

To quantify the extent to which caves were resource limited I would need to make the assumption, untested within my experiments, that if the main food resource outside the cave was transferred inside the cave, community density would increase. An improvement to this experiment would be to net off each section, inside and outside the cave, removing interference from drifting individuals, from upstream sources, and incorporate a temporal scale. Treatments would have identical units so as to be directly comparable. If a negative gradient (high and relatively even densities of individuals outside the cave but declining within the cave to minima at the most distant sites) was observed, it would suggest that leaf packs were randomly colonised by drift. However, if the opposite was observed it would suggest that caves are indeed CPOM limited. Finally, if similar densities were observed at all sites it would suggest that the whole system is limited, or not limited, by CPOM. It is likely that other resources and factors, not just biotic ones (Rowe and Richardson 2001), are

influencing cave communities and further experiments are needed to identify these and then quantify them and their interactions.

Conclusion

In conclusion, the positive response of invertebrate communities to the addition of a food resource within a cave suggested that caves were primarily limited with respect to food resources. The addition of algae to a pastoral system, resulted in algal densities decreasing at a faster rate within caves, but I was unable to assess whether this was because of the influence of grazing invertebrates, or a lack of light. The addition of leaves, to the leaf-depauperate pastoral system, resulted in changes in community composition, primarily an increase in numbers of shredders. Finally, the addition of leaves showed they were a superior resource to inorganic controls, suggesting that the pastoral cave and surface system was food limited, not habitat limited.

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Arachnocampa luminosa consuming a mayfly, 200 m within Hollow Hill cave

Chapter 4

Comparing energy pathways between cave and surface environments using stable isotopes

Abstract

Cave stream invertebrate communities have been characterised as resource limited with a dependence upon surface-derived energy resources, primarily FPOM and CPOM. This dependence upon surface derived energy, detritus, as well as the potential for cave aquatic communities to subsidise subterranean terrestrial communities was assessed by investigating a longitudinal gradient in a cave stream, using stable isotopes of carbon and nitrogen. Cave aquatic communities were found to be dependent on surface derived materials, with an isotopic signature similar to that of C_3 plants. Seston, benthic FPOM, and epilithon (i.e. bacterial, fungal, and diatom communities) were the most important basal resources within the cave, compared with seston, benthic FPOM, and filamentous algae outside of the cave. As found in a previous study CPOM did not appear to be readily incorporated into the food-web. Furthermore, in the absence of an alternative carbon source aquatic derived energy would seem to support subterranean terrestrial predators, such as glow-worms, harvestmen, and spiders.

Introduction

Surface - cave stream subsidies

Cave stream macroinvertebrate communities have been shown to use surface derived resources, primarily because of the absence of photosynthetic fixation of carbon within caves (Culver 1982, Death 1989, Simon et al. 2003). In other systems, variations in photic levels have been used to account for differences in basal resource production, i.e. between forested and pastoral streams (Hicks 1997, Quinn and Cooper 1997, Young and Huryn 1999, Scarsbrook et al. 2001, Quinn and Stroud 2002, Collier and Quinn 2003, Death et al. 2003). However, differences between cave and surface systems are even more pronounced, with surface streams containing a variety of resources compared to cave streams (Simon et al. 2003). Surface streams can be abundant in detritus, fine and coarse particulate organic matter (FPOM and CPOM, respectively) (Quinn 2000). These can be directly consumed as well as fuelling heterotrophic production by bacteria (Hall et al. 2000), and autotrophs, such as algae (McCutchan and Lewis 2002), which positively influence detrital levels. Within caves the autochthonous component is absent, detritus biomass depends on upstream supplies, and riparian inputs are minimal (Simon et al. 2003).

Studies have shown the importance of allochthonous coarse particulate organic matter (CPOM) to forested streams (France 1995, Quinn and Cooper 1997, Wallace et al. 1999, Scarsbrook et al. 2001, Collier and Quinn 2003, England and Rosemond 2004). However, in caves evidence suggests that basal resources with smaller individual biomass, such as imported algae and bacteria are more important to community functioning (Simon et al. 2003). Within caves these resources appear to be important even when large amounts of CPOM are present (Simon et al. 2003). CPOM may be directly consumed, broken down into FPOM, or made available through microbial films (Cummins 1974), but the direct use of CPOM may not be as high as previously assumed (Simon et al. 2003). Aquatic macroinvertebrates, feeding upon the afore mentioned basal resources, are important prey for predators, both aquatic (Winterbourn 2000) and terrestrial (Burdon and Harding 2008). This "leakiness" across stream boundaries provides important resources for aquatic and terrestrial communities (Wallace et al. 1997, Power 2001, Baxter et al. 2005). Specifically, consumer aquatic invertebrates are important resources for invertebrate predators, such as hydrobiosid

caddisflies (Winterbourn 2000), fish (Winterbourn 1978, Shave et al. 1994), and spiders and glow-worms (Pugsley 1984, Broadley and Stringer 2001).

Stable isotopes and food-webs

To construct food-webs in ecological systems a variety of techniques have been used to assign trophic position and food resource preference. Traditionally, feeding activities were inferred through feeding behaviours, morphology, observational experiments, and gut analysis (Winterbourn 2000). However, recently researchers have used stable isotope ratios, primarily Carbon (C) and Nitrogen (N) to infer energy flows and trophic position. Within stream systems stable isotopes were first used by Rounick et al. (1982), to assess the relative importance of autochthonous and allochthonous production. Subsequently, stable isotopic ratios have been routinely used to assess food-web structure (Peterson and Fry 1987, Hicks 1997, Pohlman et al. 1997, Bosley and Wainright 1999, Matsuoka et al. 2001, McCutchan and Lewis 2002, Post 2002, Simon et al. 2003, England and Rosemond 2004, Graening 2005, Stenroth et al. 2006, Kolasinski et al. 2008, McHugh et al. 2010).

Stable isotopes are powerful tools for untangling complicated food-webs, providing a time integrated average of an individual's or population's diet (Post 2002). They allow the tracking of energy, or mass flow, through ecological communities. This is possible because the sampled individual's isotopic ratio will reflect that of its food, prey, allowing an assessment of materials consumed. An individual's trophic level is assessed through variations in the isotopic signature of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). These assessments are made possible due to the predictable degree of change in $\delta^{13}C$ and $\delta^{15}N$ values between trophic levels. Energy is passed through trophic levels, such that $\delta^{13}C$ is enriched by about 1‰ and $\delta^{15}N$ by between 3 - 4‰, relative to their source (Post 2002), although values between 2 - 5‰ have been used to indicate a trophic level (Simon et al. 2003). A recent study by McHugh et al. (2010) used a change of 3.4‰ to denote a differences between a trophic level. However, Kilham et al. (2009) have shown a trophic step of a much lower value, as low as 1.0 - 1.7‰, in tropical streams suggesting trophic transfer of nitrogen is not straight forward. Thus, isotopic ratios reflect the assimilation of energy through all pathways leading to an organism (Hicks 1997, Post 2002, Fry 2006). A study by Thompson et al. (2007) suggested that above the herbivore trophic level food-webs were better characterised as a tangled web of omnivores, making traditional assessments of trophic position near

impossible and highlighting the importance of trophic omnivory (feeding across multiple trophic levels). Furthermore, where multiple carbon sources occur, and their $\delta^{13}C$ values overlap, it may be impossible to assess the relative importance of materials (Winterbourn 2000).

Studies within cave streams have revealed aquatic communities are primarily dependent upon surface derived organic carbon, with the exception of some geothermal systems (Sarbu et al. 1996, Engel 2007, Porter et al. 2009, Engel et al. *in press*). Typical cave aquatic food-webs contain three trophic levels; a detrital resource base, a second trophic level of detritivores, and a top level of predators, primarily fish; although predatory invertebrates are a potential third trophic level making fish a potential fourth trophic level (Graening and Brown 2003, Simon et al. 2003, Graening 2005). These detritus based systems are generally bottom-up controlled and despite being labelled as energy limited (Culver 1985), can support the formation of diverse food-webs (Polis et al. 1997). Simon et al. (2003) used tracers to assess uptakes of dissolved organic carbon (DOC), and found it was immobilised in biofilms and thus available to primary consumers. By the end of the experiment top predators, fish, were labelled with the tracer. Thus, the flow of surface derived carbon was traced through all levels of the cave aquatic community.

Aquatic to terrestrial subsidies

The effect of terrestrial subsidies on streams has been the subject of much research, e.g. in relation to Allen's paradox (Allen 1951, Waters 1988, Berg and Hellenthal 1992, Huryn 1996, Penczak et al. 1996, Collier et al. 2004, Wright-Stow et al. 2006). However, more recently aquatic subsidies to terrestrial environments have come under increasing scrutiny (Nakano and Murakami 2001, Malmqvist 2002, Baxter et al. 2005, Nowlin et al. 2008, Schneider et al. 2010, Wesner 2010). Aquatic insect biomass has been positively correlated with riparian spider density and biomass; particularly with respect to web building spiders, such as tetragnathids, araneids, and stiphidiids (Burdon and Harding 2008). These spiders as well as Opiliones (harvestmen) (known cannibals and predators of spiders (Sergeeva 1999)) are all found within caves. The commercially important species *Arachnocampa luminosa* (Diptera: Keroplatidae), or glow-worms feed upon aquatic insects and fall prey to arachnid predators (Pugsley 1984). Therefore, cave aquatic communities, dependent on surface derived subsidies, may supply a necessary subsidy to subterranean terrestrial communities.

The primary aim of this chapter was to assess the functioning and energy flow of cave aquatic food-webs. Firstly, the relative importance of differing basal resources to aquatic communities inside and outside of the cave was assessed. Secondly, interactions within aquatic invertebrate communities and between aquatic and terrestrial communities were assessed.

Methods

The study was carried out in a single stream-cave system; Murchies Stream. Initially, a second stream was included in the study however, a bacterial bloom in the headwaters markedly changed the system and severely depleted benthic communities (see Chapter 3 for greater details).

Samples for stable isotope analyses were collected on 23 February 2010, at four sites along the surface-cave stream continuum (+24 m, 0 m, -32 m, and -256 m from the cave entrance). Before entering the cave Murchies stream flows through a pastoral landscape, primarily vegetated by C_3 plants with a mean $\delta^{13}C$ value of approximately -28‰ (Fry 2006). However, it is likely that C_4 plants, with a mean $\delta^{13}C$ of approximately -13‰ (Fry 2006), were also present.

Aquatic invertebrate samples were collected by sampling a wide range of habits in each reach using a kick net. Animals were sorted in the field, and kept cool until they could be frozen. Benthic FPOM was collected from the top 10 mm of the streambed using a Turkey baster. Seston was collected using a plankton net, 60 µm mesh, placed in the water column. The seston sample from -32 m was lost in transit, but as seston did not change between the other three sites it was assumed to be similar at this site. Epilithon was collected by brushing 3-5 rocks with a nylon brush and washing the scrapings into a vial. Filamentous algae were collected with forceps from the stream bed.

Terrestrial invertebrates were handpicked. Three replicate samples of each component of the food web were taken from random locations at each site; whenever possible, > 10 individuals per sample were taken to represent taxa. Samples were frozen within a few hours of collection and returned to the laboratory. In the laboratory, samples were thawed and aquatic macroinvertebrates identified to family in most cases, although several were classified to

genus under a 40x dissecting microscope. Identifications were made using keys and guides by Winterbourn (1973) and Winterbourn et al. (2000). Spiders were classed to family using Paquin et al. (2010). Invertebrate digestive tracts were removed, and *Potamopyrgus* were manually de-shelled, and their radulae removed. *Deleatidium* and *Neozephlebia* samples were combined and homogenised because of the scarcity of individuals, as were collector browser stoneflies (predominantly *Zelandoperla*) and cased caddis (primarily *Hudsonema*).

In the laboratory, basal resources were rinsed with deionised water, excluding seston and benthic FPOM, and any contaminants, such as invertebrates, were removed. All materials were placed in eppendorf vials and dried at 45°C for a minimum of five days. Invertebrate isotope samples generally contained more than one individual, enabling the range of likely isotopic signatures to be captured. Samples were ground with a glass rod or a mortar and pestle, and returned to the drying oven. All basal resource samples were acidified with 30% HCl, to remove inorganic carbon, which may have been present in the form of limestone (Simon et al. 2003). Acid is not thought to significantly alter carbon and nitrogen values (Bosley and Wainright 1999, Kolasinski et al. 2008). Lipids were not removed from samples as they can affect nitrogen values (Post et al. 2007). Furthermore normalization equations were not deemed necessary as consumers and end members $\delta^{13}C$ values were < 10 – 12‰ (Post et al. 2007). Samples were weighted, placed in tin capsules (8 x 5 mm, OEA Laboratories Ltd, Callington, Cornwall, UK) and sent to the Cornell Isotope Laboratory (COIL: www.cosbil.com) for analysis. Analyses were preformed on a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyser. The overall standard deviation for the internal MINK standard (animal) was 0.17‰ for $\delta^{15}N$ and 0.13‰ for $\delta^{13}C$. A chemical Methionine standard, obtained from amplitudes of 250 mV and 13000 mV, produced an error associated with linearity of 0.24‰ for ^{15}N and 0.13‰ for $\delta^{13}C$.

Delta values were calculated using the formula: $\delta X\ (‰) = ((R_{sample}/R_{standard}) - 1) \times 1000$

To infer feeding a shift in $\delta^{13}C$ of ~1‰ was used, while a shift in $\delta^{15}N$ of ~3.4‰ was used, in accordance with McHugh et al. (2010). Mixing models were not deemed necessary because of the similarity of $\delta^{13}C$ values, suggesting limited variation in carbon sources.

Results

Similar basal resources were found at all sites, with the exception of filamentous algae, which were only found outside the cave (Table 4.1). Similar taxa were found at all sites, with the exception of glow-worms, which were only collected from the two deeper cave sites, and crayfish (*Paranephrops planifrons*), which were only collected from a single site inside the cave (-32 m). Thus, the range of trophic levels remained constant along the stream continuum despite the range of carbon supply becoming restricted.

Longitudinal gradients

At all sites benthic FPOM and seston had similar $\delta^{13}C$ and $\delta^{15}N$ values, suggesting the two resources contained the same material, imported from outside the cave system (Table 4.1). Interestingly, the signature of epilithon (i.e. bacterial, fungal, and diatom communities) changed between the outside and inside of the cave, suggesting a change in the composition of epilithon. Furthermore, epilithon appeared to be incorporated into the food-web at sites inside the cave but not at upstream surface sites. CPOM had different $\delta^{15}N$ values between sites, but also had different $\delta^{13}C$ values, indicating different sources of CPOM (Table 4.1). $\delta^{13}C$ values of CPOM indicated that it may be incorporated by aquatic benthic invertebrate consumers at the deep cave site (-256 m), but was not primarily incorporated into the food-web at other sites. Moss was only found outside the cave and had a completely different $\delta^{13}C$ value, around -37‰, suggesting it was not incorporated in the food-web (Table 4.1).

Across all sites combined *Deleatidium* and *Neozephlebia* individuals had similar $\delta^{13}C$ signatures, approximately equal to C_3 plants. *Deleatidium* and *Neozephlebia* samples had similar $\delta^{15}N$ values at +24 m, 0 m, and -256 m, approximately one trophic level greater than benthic FPOM (Table 4.1). However, *Deleatidium* and *Neozephlebia* at site -32 m had a lower $\delta^{15}N$ value, only ~ 1‰ higher than benthic FPOM, the most deplete basal resource measured. Enrichment in the order of ~ 1‰ is not generally considered to indicate a trophic level. This suggests that *Deleatidium* and *Neozephlebia* at this site may have been processing an unmeasured resource. *Acanthophlebia* had similar $\delta^{13}C$ values between sites, but different $\delta^{15}N$ values. Site -32 m had a lower $\delta^{15}N$ value, suggesting primary incorporation of benthic FPOM, while at site 0 m *Acanthophlebia* had a more enriched $\delta^{15}N$ value, suggesting primary incorporation of epilithon. *Potamopyrgus* $\delta^{13}C$ values slightly decreased within the cave, while their $\delta^{15}N$ values became slightly elevated (Table 4.1.). This may have been due to a

switch in food resources, from filamentous algae, in the surface stream, to epilithon (i.e. bacterial, fungal, and diatom communities), within the cave. Collector-browser stoneflies had similar $\delta^{13}C$ values at site -32 m and -256 m, enriched $\delta^{15}N$ values at the entrance, relative to sites -256 m and -32 m, and were unmeasured, as none were collected, at +24 m. Stoneflies $\delta^{15}N$ value at site -32 m and -256 m suggested the consumption of benthic FPOM. While at 0 m stoneflies appeared to primarily incorporate epilithon, although it is possible that some benthic FPOM was incorporated to their diet. The $\delta^{15}N$ signature of the predatory caddis Hydrobiosidae was not markedly higher than any measured invertebrate prey, presumably because of trophic omnivory (feeding across multiple trophic levels, i.e. basal resources and consumers) (Hicks 1997, Winterbourn 2000), or the presence of an unmeasured resource.

Opiliones (Harvestmen) had similar $\delta^{13}C$ and $\delta^{15}N$ values at -32 m and -256 m, suggesting primary incorporation of aquatic derived energy, such as *Aoteapsyche*, Hydrobiosidae, mayflies, and stoneflies (Table 4.1). Glow-worms may have been consumed by Opiliones at -256 m, but did not appear to be at -32 m, even though Opiliones were present. Lower $\delta^{13}C$ and $\delta^{15}N$ values at site 0 m were observed in Opiliones, and potentially result from their consumption of an unmeasured terrestrial source, as the $\delta^{13}C$ value of Opiliones at this site was lower than any measured aquatic source.

Tetragnathids had similar $\delta^{13}C$ values across sites (Table 4.1). Tetragnathids were not collected from site -32 m. A lower $\delta^{15}N$ value, relative to 0 m and -128 m, was observed at site +24 m, and was equated to the lower $\delta^{15}N$ value of mayflies at this site, primary prey of tetragnathids (Burdon and Harding 2008).

Araneidae had similar $\delta^{13}C$ values at sites +24 m and -32 m (Table 4.1). However, a larger $\delta^{15}N$ range was observed at site -32 m. This suggested that spiders at this site were incorporating energy from mayflies and caddisflies, while outside the cave they only appeared to incorporate energy from mayflies.

Table 4.1. Basal resource and invertebrate carbon and nitrogen ratios, at four sites along the stream continuum. Distances are in relation to the cave entrance. Values in brackets are standard error bars. Some sites do not have error bars because of the scarcity of individuals.

	+24 m	0 m	-32 m	-256 m
Epilithon				
$\delta^{13}C‰$	-20.43(0.42)	-27.66(0.31)	-25.85(0.09)	-28.79(0.05)
$\delta^{15}N‰$	3.49(0.42)	4.3(0.29)	4.4(0.88)	4.3(0.50)
Benthic FPOM				
$\delta^{13}C‰$	-28.2(0.22)	-29(1.46)	-27.88(0.07)	-27.88(0.19)
$\delta^{15}N‰$	2.21(0.06)	3.14(1.06)	2.33(0.15)	2.65(0.30)
Seston				
$\delta^{13}C‰$	-28(0.07)	-27.99(0.62)		-28.67(0.48)
$\delta^{15}N‰$	2.92	2.52(0.14)		2.42(0.13)
CPOM				
$\delta^{13}C‰$		-24.6(2.36)	-35.51	-29.69(0.62)
$\delta^{15}N‰$		2.75(0.38)	5.02	3.07(0.21)
Filamentous algae				
$\delta^{13}C‰$	-28.92(0.56)			
$\delta^{15}N‰$	3.22(1.25)			
Moss				
$\delta^{13}C‰$	-37.34(0.15)			
$\delta^{15}N‰$	3.7(0.39)			
Potamopyrgus spp.				
$\delta^{13}C‰$	-25.68(0.15)	-25.9(0.69)	-26.37(0.11)	-27.06
$\delta^{15}N‰$	6.14(0.10)	6.74(0.02)	7.07(0.17)	7.38
Aoteapsyche spp.				
$\delta^{13}C‰$	-27.71(0.39)	-27.6(0.18)	-27.46	-28.53(0.10)
$\delta^{15}N‰$	5.98(0.67)	5.05(0.54)	4.81	5.57(1.07)
Deleatidium and *Neozephlebia* spp.*				
$\delta^{13}C‰$	-27.02(0.79)	-27.61(0.61)	-27.29	-27.24(0.10)
$\delta^{15}N‰$	3.76(0.06)	4.35(0.58)	2.79	4.74(0.41)
Acanthophlebia				
$\delta^{13}C‰$		-26.34	-27.26(0.11)	
$\delta^{15}N‰$		7.77	4.12(0.10)	
Stonefly (predominantly *Zelandoperla*)				
$\delta^{13}C‰$		-26.98	-27(0.11)	-27.14
$\delta^{15}N‰$		6.73	3.66(0.27)	3.87
Hydrobiosis spp.				
$\delta^{13}C‰$	-26.87(0.27)			-27.92
$\delta^{15}N‰$	6.01(0.61)			5.21
Stiphidiidae				
$\delta^{13}C‰$	-26.59	-27.99(1.12)	-26.78(0.35)	-26.68
$\delta^{15}N‰$	8.46	5.77(2.71)	8.13(0.30)	6.81
Tetragnathidae				
$\delta^{13}C‰$	-27.03(0.33)	-26.33(0.51)		-27.02(0.05)
$\delta^{15}N‰$	6.33(0.23)	7.86(0.58)		7.31(0.05)
Araneidae				
$\delta^{13}C‰$	-26.37		-26.79(0.25)	
$\delta^{15}N‰$	6.54		7.52(0.89)	
Opiliones				
$\delta^{13}C‰$		-29.53(2.31)	-27.43	-27.05
$\delta^{15}N‰$		4.29(0.02)	7.04	8.48
Arachnocampa luminosa				
$\delta^{13}C‰$			-27.17(0.38)	-28.06
$\delta^{15}N‰$			6.97(0.09)	4.82

* *Deleatidium* spp. and *Neozephlebia* spp. were combined and homogenised because of the scarcity of individuals

Stiphidiidae had similar $\delta^{13}C$ but different $\delta^{15}N$ values at sites +24 m, -32 m, and -256 m, however variation in $\delta^{13}C$ and $\delta^{15}N$ values occurred at 0 m, relative to the other sites (Table 4.1). The $\delta^{15}N$ value of Stiphidiidae at 0 m ranged from ~3 to ~8.5‰ $\delta^{15}N$, and may have been due to an unmeasured terrestrial prey resource. At +24 m stiphidiids appeared to assimilate energy from *Aoteapsyche*, Hydrobiosidae, and mayflies; although Stiphidiidae could also have incorporated energy from tetragnathids and araneids. At site -32 m *Aoteapsyche*, mayflies, and stoneflies may have been incorporated into stiphidiid diets. However, at -256 m slightly lower $\delta^{15}N$ values were observed, relative to +24 m and -32 m, suggesting incorporation of *Aoteapsyche*, Hydrobiosidae, mayflies, stoneflies, and possibly glow-worms to stiphidiid diets.

Glow-worms were only found within the cave. They had similar $\delta^{13}C$ values throughout, but showed variation in $\delta^{15}N$ values. Glow-worms at -32 m were ~2‰ higher in $\delta^{15}N$ than glow-worms at -256 m. Glow-worms at -32 m potentially incorporated energy from *Aoteapsyche*, mayflies, and stoneflies. While at -256 m the most likely potential prey, stoneflies, were only ~1‰ $\delta^{15}N$ lower, which is not traditionally enough to infer energy transfer. This suggests the possibility of unmeasured food resources at the deep cave site. Glow-worms obtained at -256 m were predominantly early instar, and potentially had been unable to utilise aquatic derived food resources, because of their size, or a decline in aquatic invertebrate abundance.

Surface stream food-web

At this site (+24 m) aquatic invertebrate communities appeared primarily reliant upon the basal resources; filamentous algae, seston, and benthic FPOM (Figure 4.1). Both seston and benthic FPOM potentially contained filamentous algae. Epilithon and moss did not appear to be incorporated by the sampled invertebrates, because of their outlying carbon signatures. Combined *Deleatidium* and *Neozephlebia* were assumed to primarily incorporate benthic FPOM, *Aoteapsyche* primarily incorporated seston, and *Potamopyrgus* primarily incorporated filamentous algae (Winterbourn 2000). *Archichauliodes diversus* (toebiters) and hydrobiosids appeared to primarily incorporate energy from *Deleatidium* and *Neozephlebia*, although they could have incorporated filamentous algae (Winterbourn 2000). Terrestrial predators, tetragnathids, araneids, and an unknown spider also appeared to predate on mayflies. The predatory aquatic flatworm (Platyhelminthes) may have preyed upon all aquatic invertebrates. The top troglophile predator, Stiphidiidae, appeared to predate araneids, tetragnathids, and the aquatic invertebrates *Aoteapsyche*, Hydrobiosidae, *Deleatidium* and

Neozephlebia.

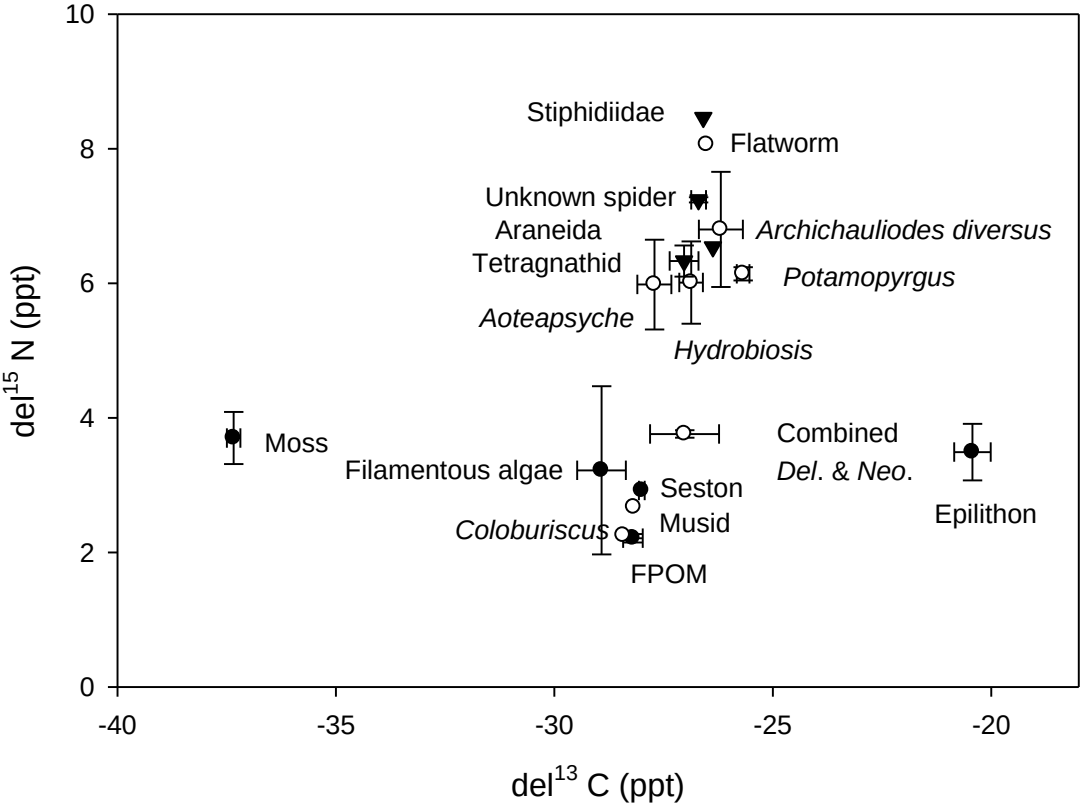

Figure 4.1. Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ values between different components of the food-web at the upstream site (+24 m) (Error bars are ± 1 standard error). Sites with no error bars did not have enough material for more than one sample. Most aquatic samples contained more than one individual. FPOM is benthic FPOM. Closed circles represent basal resources, open circles represent aquatic invertebrates, and closed triangles represent non-aquatic organisms.

Cave entrance

At this site (0 m) aquatic invertebrate communities appeared primarily reliant upon the basal resources; benthic FPOM, seston, and epilithon (Figure 4.2). *Deleatidium* and *Neozephlebia* seemed to primarily graze benthic FPOM, while *Aoteapsyche* potentially filtered both epilithon and FPOM (Winterbourn 2000). Collector browser stoneflies, cased caddis (primarily *Hudsonema*), and gastropods (*Potamopyrgus*) appeared to switch from feeding on filamentous algae at the surface stream site to epilithon within the cave. Although *Hudsonema* is an omnivore and may have also been feeding on *Aoteapsyche*, *Deleatidium* and *Neozephlebia* (Winterbourn 2000). The top predators were tetragnathids (appearing to

prey on *Aoteapsyche*, *Deleatidium* and *Neozephlebia*) and stiphidiids (appearing to prey on *Aoteapsyche*, all mayfly taxa, and Opiliones). Opiliones were potentially feeding upon an unmeasured prey, possibly of terrestrial origin.

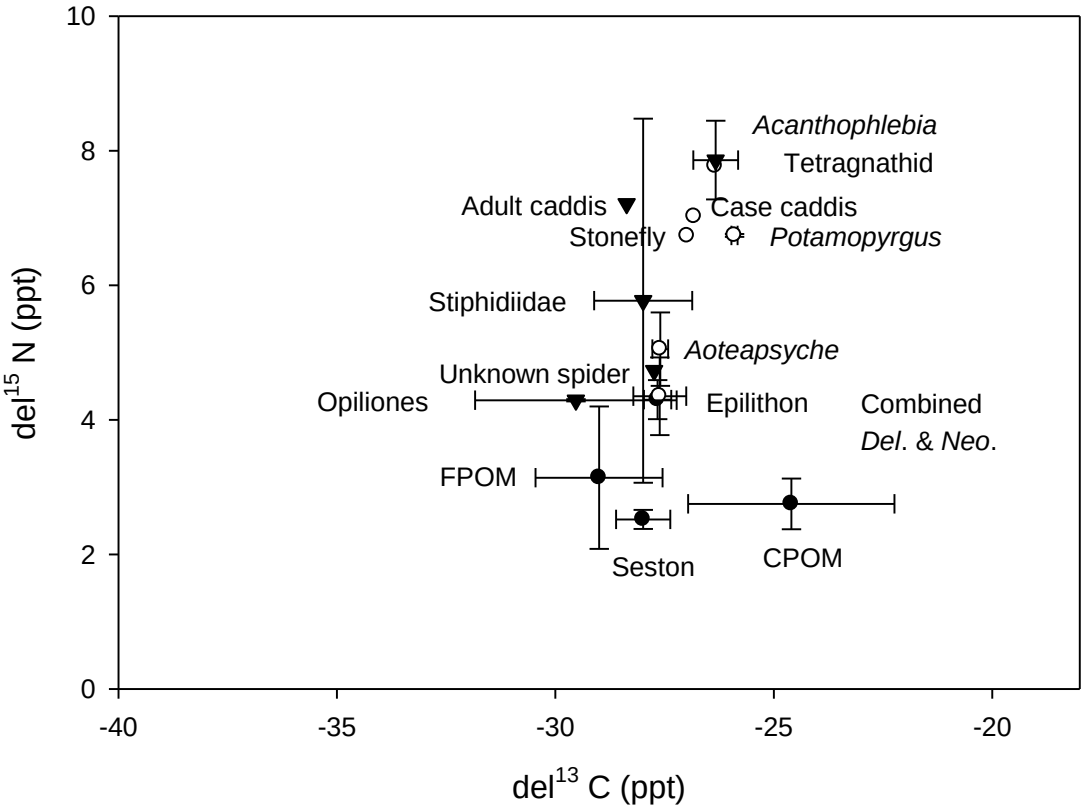

Figure 4.2. Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ values between different components of the food-web at the entrance site (0 m) (Error bars are ± 1 standard error). Sites with no error bars did not have enough material for more than one sample. Most aquatic samples contained more than one individual. FPOM is benthic FPOM. Closed circles represent basal resources, open circles represent aquatic invertebrates, and closed triangles represent non-aquatic organisms.

Cave food-web

Within the cave (-32 m) the majority of aquatic invertebrates appeared reliant upon benthic FPOM and seston (Figure 4.3). However, *Potamopyrgus* appeared to primarily feed on epilithon and benthic FPOM. Crayfish were potentially predating *Potamopyrgus* and other aquatic invertebrates, as well as grazing basal resources. There was little variation in the $\delta^{15}N$

value of terrestrial predators, suggesting exclusive predation on aquatic invertebrates, excluding *Potamopyrgus* and crayfish.

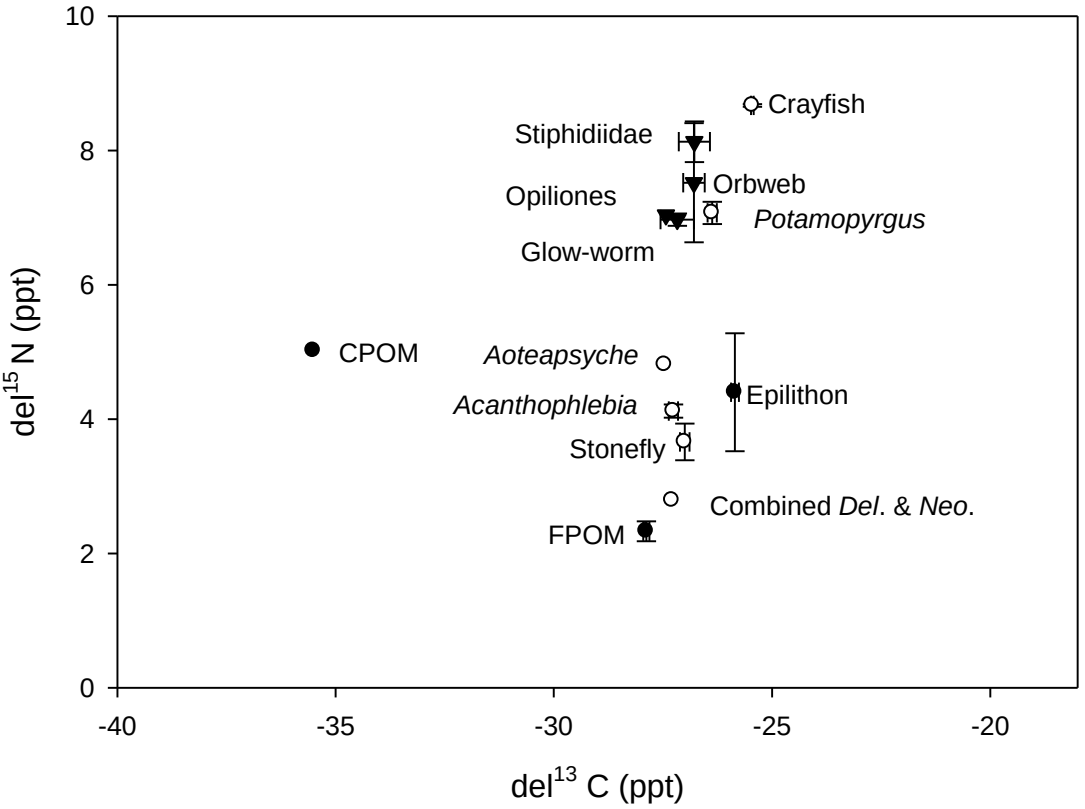

Figure 4.3. Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ values between different components of the food-web at the cave site (-32 m) (Error bars are ± 1 standard error). Sites with no error bars did not have enough material for more than one sample. Most aquatic samples contained more than one individual. FPOM is benthic FPOM. Closed circles represent basal resources, open circles represent aquatic invertebrates, and closed triangles represent non-aquatic organisms.

Cave food-web

Further within the cave (site -256 m) the majority of aquatic invertebrate consumers remained primarily dependent on seston and benthic FPOM, except for *Potamopyrgus*, which appeared to primarily feed on epilithon (Figure 4.4). Terrestrial spiders and Opiliones appeared to rely primarily upon aquatic prey, but may have preyed on glow-worms. Surprisingly, glow-worms had a lower $\delta^{15}N$ value than most aquatic prey (except stoneflies), suggesting that they may have switched to an unmeasured source, possibly a small dipteran (e.g. chironomid adults).

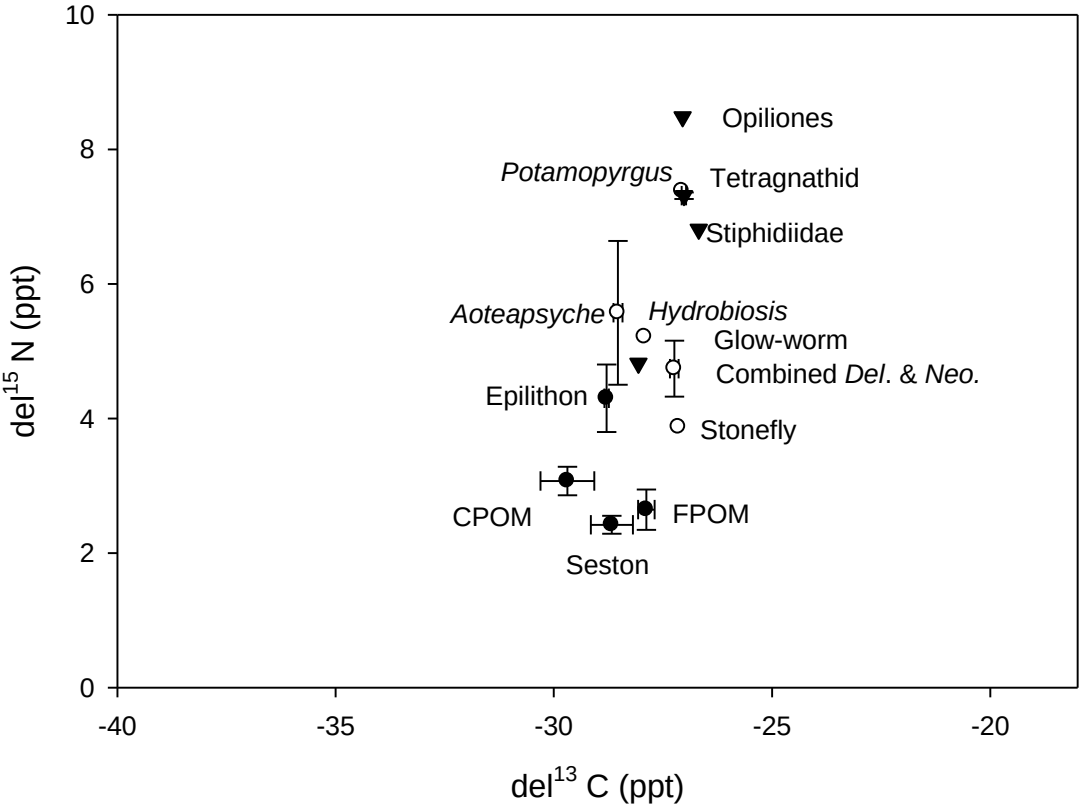

Figure 4.4. Biplot of mean $\delta^{13}C$ and $\delta^{15}N$ values between different components of the food-web at the cave site (-256 m) (Error bars are ± 1 standard error). Sites with no error bars did not have enough material for more than one sample. Most aquatic samples contained more than one individual. FPOM is benthic FPOM. Closed circles represent basal resources, open circles represent aquatic invertebrates, and closed triangles represent non-aquatic organisms.

Discussion

There was minimal change in invertebrate $\delta^{13}C$ values between the surface stream community and inside the cave, suggesting that this cave aquatic community was dependent on surface imported material. The overall carbon signature of the cave community was similar to that produced by photosynthetic fixation of atmospheric carbon by C_3 plants; a mean $\delta^{13}C$ value of approximately -28‰ (Winterbourn 2000, Fry 2006). Seston, benthic FPOM, and epilithon appeared to be the most important basal resources supporting the cave invertebrate community. Seston and benthic FPOM had similar $\delta^{13}C$ and $\delta^{15}N$ values within and across sites, presumably because benthic FPOM was predominantly composed of surface

derived seston. Epilithon did not appear to be incorporated into the food-web outside the cave, probably because of an abundance of filamentous algae. The isotopic signal of epilithon changed between cave and surface sites, presumably because of a lack of photosynthetic producers within caves. However, within the cave epilithon became an important primary resource, congruent with the scarcity of autotrophs.

The changing importance of epilithon, as a basal resource, was reflected in the gastropod, *Potamopyrgus*, which appeared to shift from grazing filamentous algae, on the surface, to grazing epilithon, within the cave (Rounick et al. 1982, Hicks 1997). *Potamopyrgus* within the cave showed unusually elevated $\delta^{15}N$ values, although these were still within the bounds of one trophic level (~3.4‰) of epilithon. These unusually high values are potentially explained by a high efficiency of food to tissue conversion (Ricciardi and Whoriskey 2004). Also, *Potamopyrgus* excreta is known to contain high ammonium levels, and in systems where they are the dominant consumer of basal resources (average density of 6724 m^2 and 1198 m^2 outside and inside the cave, respectively, in this study) their excretion accounted for the majority of ammonium present (Hall et al. 2003). Thus, a high biofilm $\delta^{15}N$ signature relative to other basal resources was observed, as well as an increased $\delta^{15}N$ signature for *Potamopyrgus*. *Potamopyrgus* were also potentially consuming conspecific faecal pellets, resulting in a net $\delta^{15}N$ enrichment of the *Potamopyrgus* population (Kilham et al. 2009). CPOM did not appear to be directly incorporated into the food-web at any of the sites, except possibly -256 m. However, CPOM may play an important role in the formation of FPOM or as substrate for the growth of epilithon.

My results suggested that some aquatic taxa (e.g. *Acanthophlebia*) varied their primary food source between sites within the cave, potentially because of the relative abundance of resources. Terrestrial taxa also displayed a longitudinal shift in prey taxa. Surface dwelling spiders appeared to incorporate both mayflies and caddisflies, while inside the cave some spiders primarily preyed on mayflies, while others primarily preyed on $\delta^{15}N$ enriched *Aoteapsyche*. This may have been a response to prey availability.

Hydrobiosid larvae found within the cave were predominantly late instars, and consequently expected to have a higher $\delta^{15}N$ value, because they are recognised as predatory invertebrates (Winterbourn 2000). However, I observed a lower $\delta^{15}N$ value, relative to their hypothesised prey, which was possibly indicative of these hydrobiosids directly incorporating basal resources into their diet (Winterbourn 2000). This trophic omnivory, which has been seen in

other studies (Hicks 1997), may potentially occur because of a decrease in prey abundance. Crayfish are known to be omnivores and I observed a high $\delta^{15}N$ value in crayfish, suggesting that in this cave they predate/scavenge macroinvertebrates as well as basal resources (Hicks 1997, Stenroth et al. 2006). Eels were also observed at all sites in this study and seen to predate crayfish and are known crayfish predators (Shave et al. 1994).

Spiders and Glow-worms within the cave appeared to feed upon aquatic invertebrates (Pugsley 1984, Malmqvist 2002, Burdon and Harding 2008). Therefore, imported surface energy subsidises the cave aquatic community, which in turn fuels the cave terrestrial invertebrate communities. It is highly probable that the terrestrial cave community has higher diversity and is extended further into caves via energy derived from cave streams. There was also evidence suggesting predation, as well as cannibalism, occurred between troglophiles, as noted by Pugsley (1984). However, the magnitude and effect of this are not known, and need to be further investigated for a greater understanding to be achieved.

In conclusion, both aquatic and terrestrial cave communities were supported by surface derived organic materials. Seston, benthic FPOM, and epilithon appeared to be the most important basal resources inside the cave, compared to seston, benthic FPOM, and filamentous algae outside the cave. Outside of the cave the, presumably, autotrophic epilithon was not incorporated into the food web, but within the cave the non-autotrophic epilithon (i.e. bacteria and fungi) became important. As found in a previous study by Simon et al. (2003) CPOM did not appear to be directly incorporated into the food-web. Secondly, imported aquatic energy was ultimately found to support terrestrial predators within the cave, such as glow-worms and spiders, presumably via predation of aquatic winged adults. Finally, aquatic and non-aquatic cave communities, including the iconic glow-worm populations, dependence upon surface processes intricately links their health to that of the surface system, tightly linking them to land-use changes.

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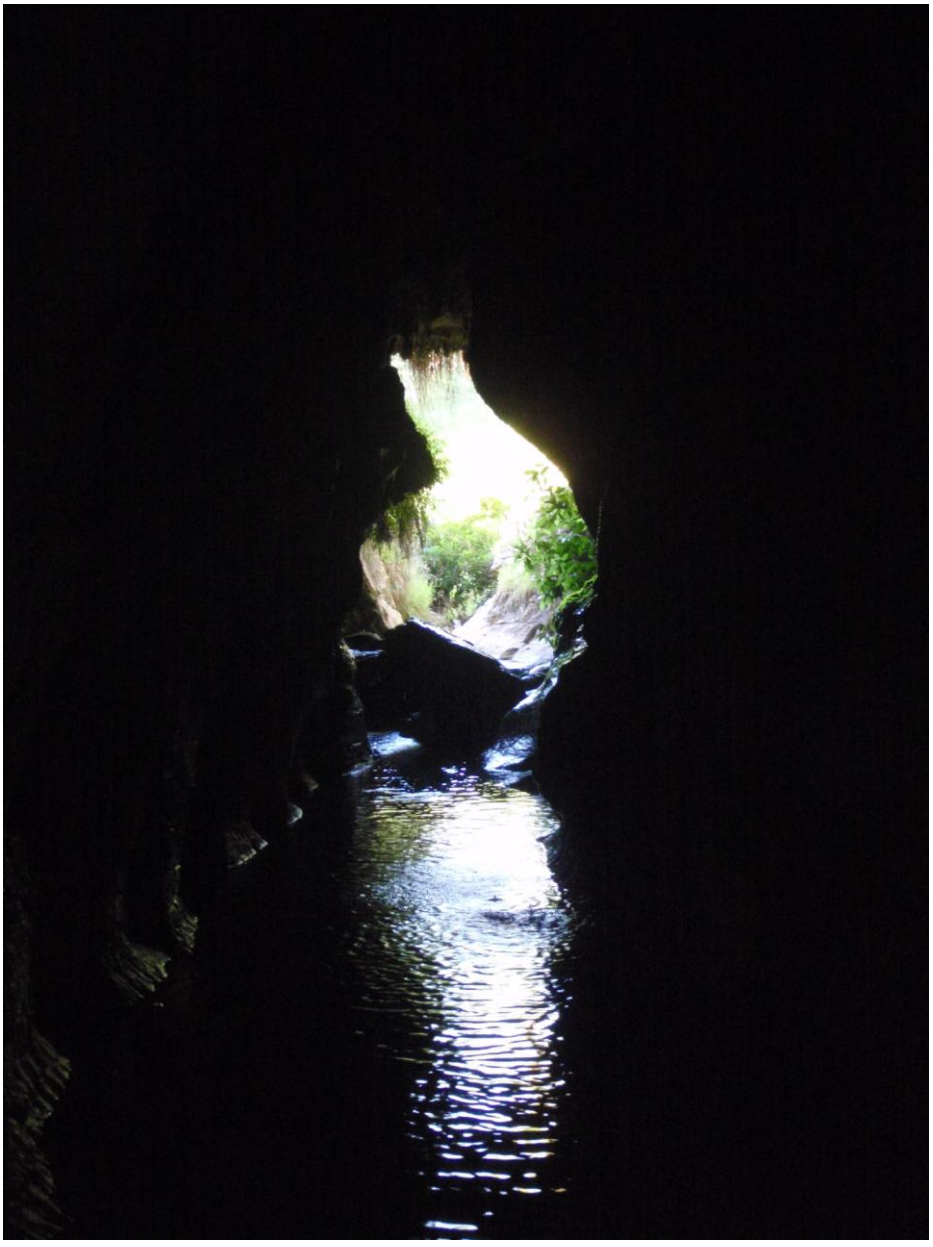

Exiting Murchies cave

Chapter 5

Synthesis: the structuring of aquatic macroinvertebrate communities within cave streams

The primary aim of my study was to quantify changes in aquatic invertebrate communities that occur as surface streams flow into caves and investigate the fundamental drivers of cave ecosystems. Not surprisingly, noticeable differences were observed between cave and surface systems, physically and ecologically. Firstly, cave aquatic macroinvertebrate communities seemed heavily reliant upon drift for recolonisation and importation and replenishment of surface derived energy (compared to surface communities' reliance upon detrital and autochthonous energy, reproduction, and drift) (Figure 5.1). Secondly, cave communities were a depauperate sub-set of surface communities, with no cave specialists observed within these systems. The lack of cave specialists within this study was not surprising as only the first 256 meters of cave streams were sampled. Studies which have identified cave specialists have found them in streams originating underground, or much further within caves (Gibert et al. 1994, Gunn et al. 2000). Physically, light intensity, chlorophyll-*a* concentration, and CPOM bioimass decreased significantly within caves, while water temperature, dissolved oxygen, and stream width, depth, and velocity remained unchanged.

Several invertebrate functional feeding groups were noticeably absent within my caves. For example, algal piercers (predominantly *Oxyethira* spp.) and predators (predominantly *Hydrobiosis* spp.) were rare, while a few groups increased (predominantly collector-browser mayflies) within caves. Upstream land-use appeared to be biasing cave communities, presumably because of unique abiotic and biotic factors. The agricultural systems had high levels of surface autochthony leading to increased algal exportation to caves, while the

forested stream had increased allochthonous inputs leading to increased CPOM inputs to cave systems. Furthermore, variations in surface land-uses led to the formation of unique surface aquatic macroinvertebrate communities, leading to variation in the composition and density of cave aquatic macroinvertebrate communities of differing land-uses. These findings demonstrate the importance of surface terrestrial and aquatic systems to cave aquatic macroinvertebrate communities (Figure 5.1).

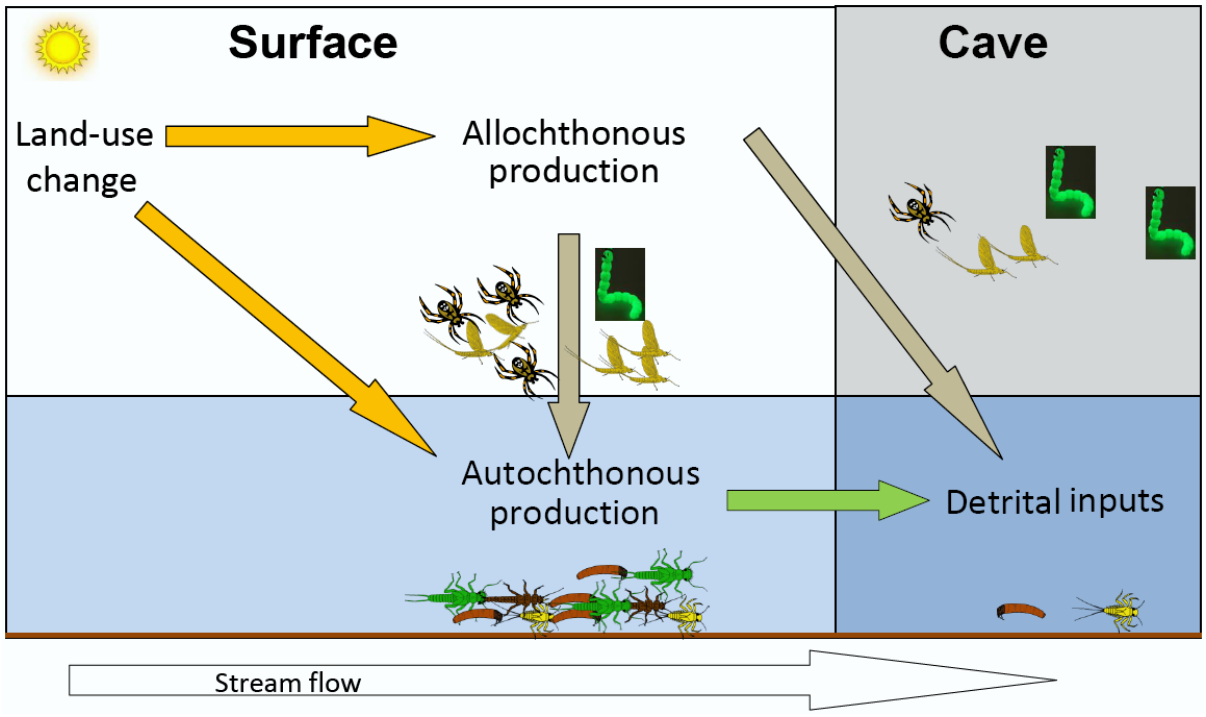

Figure 5.1. Surface land-use changes affect abiotic and biotic factors causing changes to allochthonous, autochthonous, detrital resources, and surface stream invertebrate communities which in turn exert a degree of control over cave communities. Cave aquatic invertebrates communities were fuelled by surface derived resources, and in turn supported terrestrial predators, within caves. Aquatic invertebrates were a subset of surface communities and appeared to be structured by a lack of resources.

Resource availability structures communities

As expected benthic taxa diversity and density decreased within the caves relative to surface sites. Presumably this was because of increasing environmental harshness, primarily the loss of solar energy, leaving cave communities dependent upon imported surface derived resources.

The majority of taxa densities within cave communities appeared to be explained by the density of surface communities and drift rates. However, a few taxa were present upstream of the caves but absent from within the caves, suggesting that an organism's physical and behavioural adaptations, as well as resource/environmental gradients were important for within cave survival. Surprisingly, mayfly and *Austrosimulium* densities increased within caves. Presumably this was because of the relative increase in resource availability, or a decrease in predators. Several taxa were noticeably absent, or very rare within caves, including the algal piercer *Oxyethira*, presumably because of a lack of filamentous algae on which it feeds (Quinn and Hickey 1990). Elmidae also appeared to find the cave environment unsuitable, and had low benthic density but increased drift density relative to surface sites. Chironomids too were rarely present within the caves. Most of those outside the cave were tube-dwelling species and unlikely to drift, and it appears that few adults were attracted into the caves to lay eggs. Predatory invertebrates (primarily *Hydrobiosis* spp.) were presumably not found within the caves because of the decrease in total prey density, or increasingly harsh abiotic conditions (e.g. a preponderance of bedrock lacking refuges for predators) making predation difficult (Peckarsky 1983). Death (1989) obtained similar results, except that mayflies were less abundant in the cave he studied.

Macroinvertebrate drift densities remained constant across the ecotone (environmental gradient), by virtue of increased drift propensity (drift density / benthic density) (Figure 5.2a). Presumably individuals within caves increased their drift in response to resource limitations (Death 1988) (Figure 5.2b). Furthermore, reproduction within cave aquatic communities has not been recorded, leaving subterranean aquatic communities reliant on surface derived individuals for recruitment (Figure 5.2b). Drift outside caves may be due to a range of factors (stochastic or otherwise), including possible responses to increased competition or predation, in accordance with Peckarsky's (1983) harsh benign model (Figure 1.1), or perhaps a relative lack of resources (Figure 5.2). Harsh environmental conditions within caves, presumably

lead to cave macroinvertebrate communities having a higher turnover rate than surface communities, i.e. an individual was likely to stay on the benthos for a shorter time within a cave because of limited resources, whereas outside of the cave an individual may settle sooner and remain longer (Death 1988) (Figure 5.2b).

Environmental gradients provide useful contexts for addressing key questions in ecological theory (Agrawal et al. 2007). They provide the opportunity for natural experiments where the strength of biotic interactions and other components of community assembly can be investigated along abiotic gradients. Within my study system caves were assumed to be harsh environments, relative to the surface community, primarily because of the lack of light; leading to a lack of photosynthetic production and lower temperatures, resulting in dependence upon imported surface derived resources. This in-turn suggests, in accordance with Peckarsky (1983) and Grieg (2008, unpublished data), that abiotic interactions will structure cave communities, leading to noticeably different densities of predators, as was seen in my study.

The increase in mayfly density within caves combined with their increased drift rates suggested that drift, as opposed to early emergence, was their preferred method of leaving an unsatisfactory environment. Within an unsuitable habitat some orders, such as mayflies, can speed up development, to a winged adult, thus leaving an unsuitable environment, while other orders, such as caddis, cannot (Dahl and Peckarsky 2003). Within other harsh systems lower densities of predators have been observed, indicating sub-optimal habitat and foraging conditions, or a lack of colonisation ability (Grieg 2008). Evidence from my thesis suggests the harsh-benign theory is relevant between surface and cave systems, which appear *prima facie* to be structured by abitoic factors, primarily resource limitation.

Source-sink dynamics

Source-sink interactions occur when individuals from highly productive source habitats move into less productive sink habitats, where mortality is greater than reproduction (Pulliam 1988, Pulliam and Danielson 1991, Leibold et al. 2004, Holyoak et al. 2005). Source-sink dynamics in caves appear driven by drift, active or passive, and were almost certainly occurring, as cave communities were a sub-set of surface communities. It was unknown whether reproduction occurred within caves; however, a lack of reproduction within caves would lead to further depletion of cave communities (Figure 5.2).

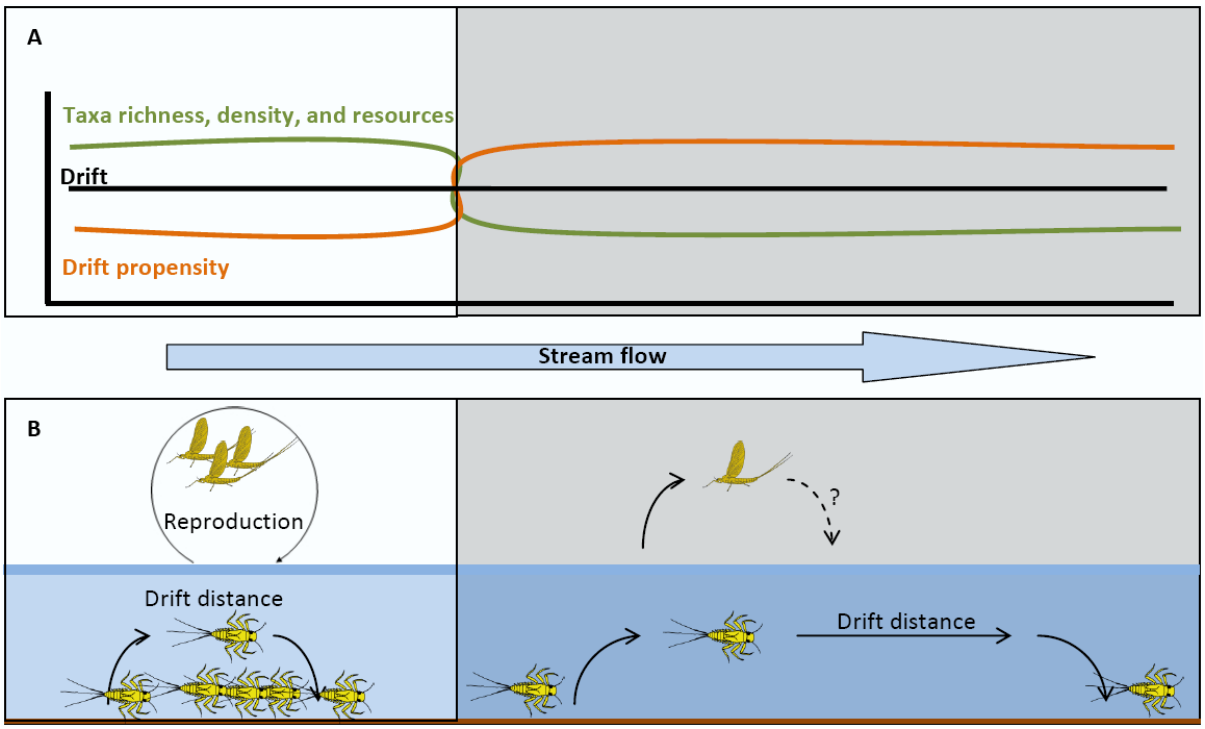

Figure 5.2. Conceptual diagrams (A and B) showing the processes involved in invertebrate community structure across cave stream gradients; resources, drift, and reproduction. Diagram A represents observed results. Diagram B shows the hypothetical increase in drift distances within caves. Drift distances potentially increased within caves because of a lack of available resources. The mayfly within the diagram represents aquatic macroinvertebrates, while the grey region represents the cave.

Cave stream aquatic community food-webs were compiled from stable isotope and benthic density data, literature, and field observations (Figure 5.3). Isotopic analyses suggested the main difference between surface and cave communities was a switch from dependence on filamentous algae to epilithon (i.e. bacterial, fungal, and diatom communities). Increased consumption of epilithon growing upon organic matter within caves may have lead to increased relative availability of FPOM as frass.

My study showed the cave entrance community to be structured differently from the surface or cave sites. Within the isotope and benthic survey the entrance had lower aquatic invertebrate taxa richness and density. Predatory invertebrates were absent from the food-web

(although the omnivorous cased caddis *Hudsonema* spp. may have acted as a predator (Winterbourn 2000)), possibly because of a lack of prey or harsh abiotic factors. The lower density of individuals within the entrance zone was possibly because of, 1) increased disturbance, 2) increased drift before desensitisation to the cave environment, or 3) upstream migration out of the cave. There was evidence that some taxa (e.g. *Acanthophlebia*) switched from predominantly feeding upon epilithon at the entrance to FPOM within the cave, possibly due to changes in the availability of resources at the surface-cave ecotone. However, despite differences at the cave entrance there was surprisingly little difference between surface and cave food-webs (Figure 5.3).

Further research

My research has shown that there is enormous potential for continued research within caves, especially in relationship to their aquatic communities. Specifically, studies on: 1) aquatic invertebrate reproduction within caves, in order to further assess community structuring, 2) the possibility of chemotrophic production within cave biofilms, 3) interactions within and between terrestrial and aquatic communities (including fish) within caves. In my study some terrestrial predators had lower isotopic values than associated aquatic prey, suggesting the possibility of unidentified terrestrial, or aquatic, interactions. Furthermore, competition between spiders and glow-worms (*A. luminosa*) occurred when the former constructed webs in front of the latter, effectively intercepting winged invertebrates attracted to glow-worms; a form of kleptoparasitism, 4) Invertebrate feeding behaviour with respect to increasing resource depletion, with specific interest in omnivory, 5) seasonal and pollutant effects upon cave communities, 6) comparisons of invertebrate functioning inside and outside of caves, and 7) comparisons of invertebrate structuring and functioning within and between tourist and wild caves.

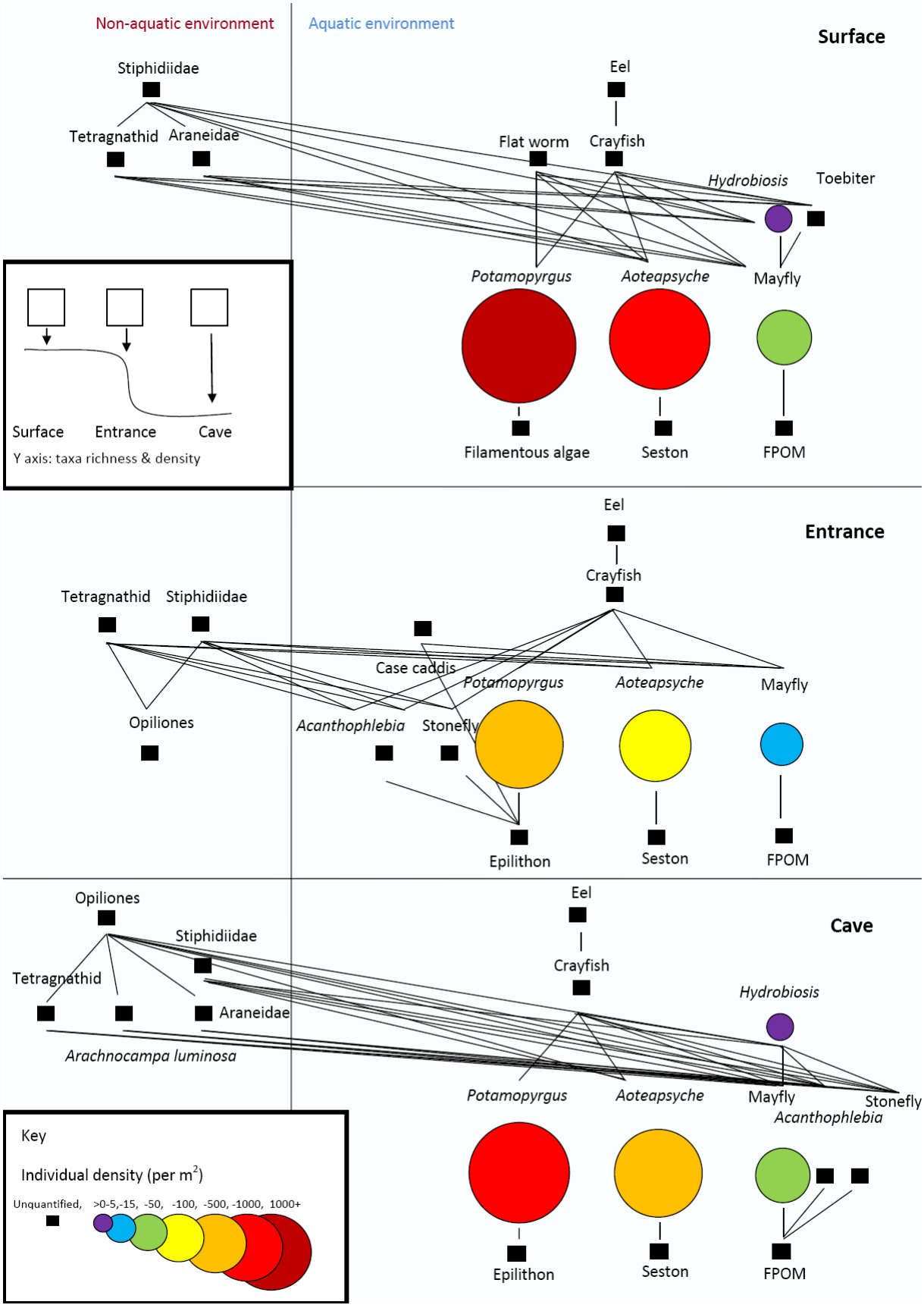
Non-aquatic environment
Aquatic environment
Surface
Stiphidiidae
Tetragnathid
Araneidae
Eel
Flat worm
Crayfish
Hydrobiosis
Toebiter
Mayfly
Potamopyrgus
Aoteapsyche
Filamentous algae
Seston
FPOM
Surface
Entrance
Cave
Y axis: taxa richness & density
Entrance
Eel
Crayfish
Tetragnathid
Stiphidiidae
Case caddis
Potamopyrgus
Aoteapsyche
Mayfly
Opiliones
Acanthophlebia
Stonefly
Epilithon
Seston
FPOM
Cave
Opiliones
Stiphidiidae
Eel
Crayfish
Tetragnathid
Hydrobiosis
Araneidae
Arachnocampa luminosa
Potamopyrgus
Aoteapsyche
Mayfly
Stonefly
Acanthophlebia
Epilithon
Seston
FPOM
Key
Individual density (per m2)
Unquantified,
>0-5,-15,
-50,
-100,
-500,
-1000,
1000+

Figure 5.3. Aquatic community food-webs constructed from stable isotope and benthic density data, literature, and field observations. Food-webs were compiled for the surface, entrance, and cave zone. The cave food-web was a combination of sites -32 m and -256 m from the isotope survey. While the entrance and surface food-webs were sites 0 m and +24 m respectively, from the isotope survey. Benthic densities for the surface web were an average of sites +32 m and +8 m; while the entrance web was from site 0 m, and the cave web was an average of sites -32 m, -128 m, and -256 m. Densities were only measured for aquatic macroinvertebrates and subsequently all other groups are labelled as unquantified. Macroinvertebrates labelled as unquantified were recorded in the isotope survey but not within the 2009 survey. FPOM was benthic derived FPOM. Mayflies were predominantly *Deleatidium* and *Neozephlebia* spp., stoneflies were predominantly *Zelandoperla* spp., and cased caddis were predominantly *Hudsonema* spp.

Management implications of my research

Light displays by the New Zealand Glow-worm, *Arachnocampa luminosa* (Diptera: Keroplatidae), within caves have led to an array of tourism operations. There is little understanding as to the effect these operations have on aquatic benthic invertebrate communities. However, the continued supply of winged aquatic insects, an important food resource, is necessary for the maintenance of healthy glow-worm populations (Pugsley 1984). To conserve and manage cave invertebrate communities, particularly with response to tourism development, a better understanding of the ecology of aquatic communities fuelling glow-worm populations is needed.

Traditionally, interactions between the environment and socio-economic behaviours of humans are outside the pure view of population and community ecology and rarely considered (Sagarin 2007). However, socio-economic behaviours of humans often lead to the strongest relative forces structuring ecological systems and must be considered in the functioning of ecosystems (Darwin et al. 1996, Sagarin 2007), i.e. active management processes.

We are now living in a world economy the destiny of which we all share. Prosperity and economic growth are occurring on a scale never conceived by earlier generations, unfortunately, so are threats to ecological systems (Andersson and Eriksson 2010). Awareness of the clash between economic and ecological systems is becoming increasingly recognised, locally as well as globally, leading to pressures on companies and governments to

limit resource exploitation (Darwin et al. 1996, Scoones 1999, Robert et al. 2002, Ayres 2004, Andersson and Eriksson 2010). Factors affecting ecosystems include, but are not limited to, land-use alterations, climate change, species invasions, and overexploitation of resources (Vitousek et al. 1997, Foley et al. 2005, MEA 2005), especially in aquatic ecosystems (Konikow 2002, Angeler 2007, Smol and Douglas 2007). These factors singly, or combined, are impacting individuals and the functioning of populations, communities, ecosystems, and the biosphere (Vitousek et al. 1997).

As previously mentioned, it would appear that aquatic invertebrates within cave streams are primarily resource limited, and what resources are available are generally surface derived. Therefore, the continued management of cave aquatic populations has to begin above the cave entrance (Graening 2005, Wood et al. 2008). This equates firstly to effective surface land-use management, e.g. riparian, waste, water abduction, sedimentation, and visitor management (to name a few), and secondly to the management of these factors within caves (Table 5.1) (Gunn et al. 2000, Sala et al. 2000). As a short term management strategy it would be possible to add food resources to cave streams to boost aquatic juveniles and therefore aquatic adults, which are glow-worm prey. But a heavy reliance upon surface communities for colonists would render this as an inappropriate long term solution, if the surface system was being degraded. Management needs to look beyond short term (Robert et al. 2002), and it is unknown what result the addition of a food resource to a cave community would produce long term. It is possible, as the results indicate, that an increase in aquatic invertebrates, and therefore glow-worm prey, would be observed, potentially leading to an increase in glow-worm abundance. However, this deserves greater study before significant levels of resources are added to cave streams.

Table 5.1. Summary of external and internal threats to subterranean ecosystems and their potential impacts, from Gunn et al. (2000).

Threat	Impact on cave		Impact on ecology
External			
Quarrying and mining	Water quantity	Volume of water	Loss and creation of aquatic habitats
		Flood hydrograph	Erosion/deposition of taxa Impact on food chains and life-cycles
		Diversion of water	Loss and creation of habitats Erosion/deposition of taxa
	Water quality	Chemistry	Highly variable
		Colour	Unknown
	Physical	Erosion	Reduction in fine sediment habitats Flushing of interstitial habitats Mobilization of organics
		Deposition	Siltation/loss of interstitial habitats Increase in fine sediment habitats Accumulation of organics
	Gaseous	Airflow and microclimate	Highly variable
Landfill/waste disposal	Water quality	Chemistry	Highly variable
		Colour	Unknown
	Gaseous	Toxic gas	Depends on solubility in water
Agriculture	Water quantity	Volume of water	Loss and creation of aquatic habitats
		Flood hydrograph	Erosion/deposition of taxa Impact on food chains and life-cycles
		Diversion of water	Loss and creation of habitats Erosion/deposition of taxa
	Water quality	Chemical, e.g. fertilizers, herbicides and pesticides	Highly variable, e.g. nutrient enrichment, change in community structure and food chains, elimination of taxa Increase in sanitary bacteria
		Biological	Reduction in fine sediment habitats
		Erosion	Flushing of interstitial habitats Mobilization of organics.
		Deposition	Siltation/loss of interstitial habitats Increase in fine sediment habitats Accumulation of organics
Groundwater abstraction	Water quantity	Reduced volume of water	Loss of aquatic habitat
	Physical	Deposition	Siltation/loss of interstitial habitats Increase in fine sediment habitats Accumulation of organics
Internal			
Caving activities (including exploration)	Water quantity	Diversion of water	Loss and creation of habitats Erosion/deposition of taxa
	Physical	Impoundment/ ponding	Interfere with dispersal and migration
		Erosion and deposition	See above for details
	Nutrient status	Organic litter (wood, etc.)	Change community structure and food chains, elimination and/or colonization of new taxa

With such large effects and unprecedented levels of change predicted (e.g. Sala et al. 2000), managers must look further and more carefully into the future, in an effort to preserve ecosystems for generations to come (Christensen et al. 1996, Vitousek et al. 1997, Sala et al. 2000, Andersson and Eriksson 2010). Cave environments are some of the oldest, unchanged, and most beautiful systems on earth; however, they are often ignored because they are rarely seen and difficult to work in. This ignorance or "out of sight out of mind attitude" is no longer acceptable (Wood et al. 2002).

Deforestation and the increase of intensive farming in New Zealand (in 2002, 71% of New Zealand's original forest, or ~14,000,000 ha, had been lost (Ewers et al. 2006)), has lead to increased pollution and runoff causing significant changes to surface aquatic communities (Harding et al. 1999, Quinn 2000, Muller et al. 2006, Ramesh et al. 2009, White et al. 2010). So far overseas aquatic cave communities have varied in response to pollution events from complete degradation (Shi et al. 2009) to relative resistantance (Gunn et al. 2000, Wood et al. 2008), and in a few cases may even have benefitted (Wood et al. 2008). There are no published studies on the effect of surface pollution to New Zealand aquatic cave communities. However, if pollution to cave streams continues a tipping point may be reached. Beyond this point cave aquatic communities may be unrecognisable, resulting in the potential loss or decrease in cave terrestrial populations, including glow-worms. Restoration of these systems is likely to be costly, time consuming, dangerous, and would only return them to an alternative stable state, as complete restoration would be near impossible. Therefore a sustainable prevention method is better than a complete restoration process. However, if a cave system is already degraded the surface aquatic system must be restored (potentially by the restoration of the surface terrestrial system), as this system supplies the energy and aquatic colonists present within cave aquatic communities.

Caves (unlike forests and grasslands) form on a geological time scale, and any degradation of their physical environment is unlikely to be repaired within our lifetime. Therefore, before decisions are made they need to be carefully and thoroughly investigated. Finally, cavers are constantly pushing boundaries and exploring areas of caves never before seen by humans (Gunn et al. 2000), and as such there is little known of their associated biotic communities. It is important that science doesn't wait until these pristine systems have been degraded before it begins to assess life in these beautiful but harsh environments.

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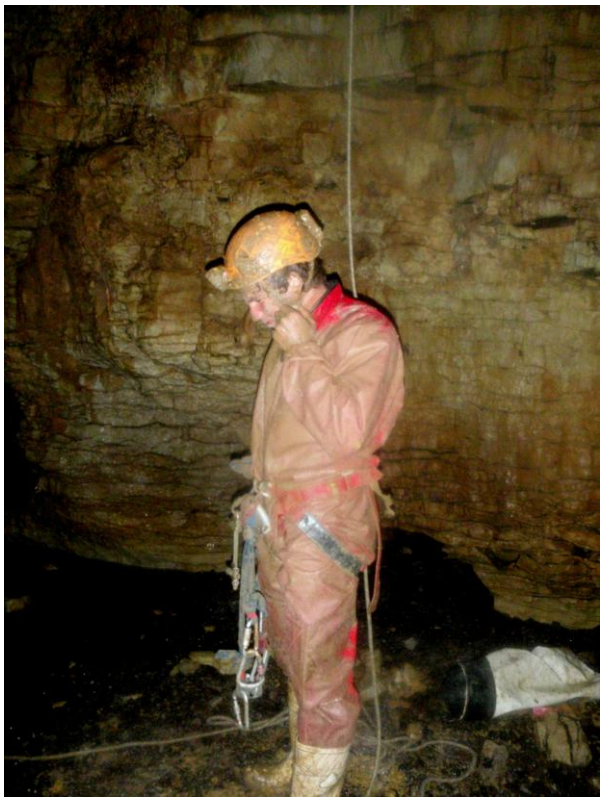

Playing in the mud